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SHOCK®

Molecular, Cellular, and Systemic
Pathobiological Aspects
and Therapeutic Approaches

Abstracts and Program
FOURTH INTERNATIONAL SHOCK CONGRESS
AND
TWENTY SECOND ANNUAL CONFERENCE ON SHOCK
Philadelphia, Pennsylvania
June 12–16, 1999

The Official Journal of

The Shock Society
The European Shock Society
The Brazilian Shock Society
The Indonesian Shock Society
The International Federation of Shock Societies, and
The Official and International Journal of the Japan Shock Society

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Molecular, Cellular, and Systemic Pathobiological Aspects and Therapeutic Approaches

OFFICIAL JOURNAL OF THE SHOCK SOCIETY, THE EUROPEAN SHOCK SOCIETY, THE BRAZILIAN SHOCK SOCIETY, THE INDONESIAN SHOCK SOCIETY, THE INTERNATIONAL FEDERATION OF SHOCK SOCIETIES, AND THE OFFICIAL AND INTERNATIONAL JOURNAL OF THE JAPAN SHOCK SOCIETY

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Summary of Symposia at the 4th International Shock Congress and the Twenty-Second Annual Conference on Shock held in Philadelphia, PA, June 12-16, 1999.

Summary of Symposia at the 7th International Cytokine Conference held in HiltonHead, SC, December 5-9, 1999

Both symposia were very successful. The attached summaries describe the State-of-the-art scientific progress being made in these vital areas. Also attached are abstracts of all the papers delivered at the Symposia.

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Fourth International Shock Congress and Twenty-Second Annual Conference on Shock Philadelphia, Pennsylvania

Saturday, June 12 to Wednesday, June 16, 1999

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SHOCK SOCIETY
FOURTH INTERNATIONAL SHOCK CONGRESS
AND
TWENTY-SECOND ANNUAL CONFERENCE ON SHOCK
PHILADELPHIA, PENNSYLVANIA
June 12-16, 1999
REPORT

The 4th International Shock Congress was held in historic Philadelphia close to Independence Hall and the Liberty Bell. The setting was outstanding. The scientific program consisted of symposia, plenary lectures, debates and poster sessions. Following are the Plenary Lectures and Symposia along with the published summary of each symposium.

PLENARY LECTURES

The Evaluation and Future Trends in the Shock Patient From the Home to the Hospital
Jean-Louis Vincent, MD,PhD

Gender Dimorphism and the Effects of Sex Steroids in the Divergent Response to Shock
Irshad H. Chaudry, PhD

Immunological Trends in the Treatment of the Shock Patient
Eugen Faist, MD

The History of the International Federation of Shock Societies
Kazuo Okada, MD,PhD

SYMPOSIA

Cytokines in Shock
Speakers: Wolfgang K. Ertel, MD; Lyle L. Moldawer, PhD; Judy A. Spitzer, PhD; Brett P. Giroir, MD

"It has been claimed that proinflammatory cytokines play major roles in the pathogenesis of various types of shock and multiple organ dysfunction syndrome following shock. Many immunological approaches to modulate those cytokines such as monoclonal antibodies, receptor antagonists and soluble receptors, have been tried experimentally but are proven to be ineffective in clinical settings. However, we now have new therapeutic tools for the modulation of cytokines such as biological control using anti-inflammatory cytokines and continuous blood purification. In this symposium, we have two papers on the role of cytokines in shock, sepsis and injury and two papers dealing with biological control again cytokine network using interleukin-10 and bactericidal/permeability-increasing protein".

Endotoxin Tolerance
Speakers: Jean-Marc Cavaillon, Dr.Sc; H.W. Löms Ziegler-Heitbrock, MD; Michael A. West, MD, PhD; John J. Spitzer, MD; Ronald G. Thurman, PhD

"Gram Negative sepsis and septic shock are common causes of critical illness among patients treated in the intensive care units. Despite improved therapeutic modalities based on better understanding of the underlying processes mortality in septic conditions remains very high. New approaches are therefore urgently needed to achieve better treatment results. Endotoxin is considered an important factor in the pathophysiology of Gram negative sepsis: both as a triggering factor during the initiating moments but also during the continued progression of the disease that is common in severe cases. Resistance to endotoxin has been demonstrated both as a constitutive and as an inducible phenomenon. Further understanding of the underlying mechanisms of endotoxin resistance should provide a basis for new modalities of treatment of Gram negative sepsis."

The Microcirculation in Shock
Speakers:Geert Schmid-Schönbein, PhD; Rosario Scalia, MD,PhD; Konrad Messmer, MD; Mark G. Clemens, PhD; Makoto Suematsu, MD,PhD

"The microcirculation has been well documented to be a target site for pathophysiological actions of endotoxin and sepsis. Endothelial activation and leukocyte adhesion have been implicated as critical steps in microvascular injury, leading to increased permeability; thrombosis; and interactions with macrophages, platelets, acute-phase responses, hematopoietic systems, and a multitude of mediators (eicosanoids, nitric oxide, cytokines, chemokines, adhesion molecules, oxyradicals, platelet activating factor, endothelin, heme oxygenase, etc.). Recent evidence indicates leukocyte-endothelial interactions and upregulation of surface adhesion molecules not only precede leukocyte infiltration, but that this process is accompanied by oxygen free radical production and parenchymal cell death. New pharmacologic interventions (heparinase III; serine protease inhibitors; trimethylsphingosine; $ONOO^-$) decrease expression of these adhesion molecules and reduce leukocyte rolling, adherence and transmigration produced by NOS inhibition and ischemia-reperfusion. NOS blockade also enhances LPS-induced hepatic microvascular failure and leukocyte accumulation; furthermore, post-ischemic hepatic microvascular changes correlate with hepatic dysfunction. Exciting new evidence involving characteristic gene expression patterns and differential roles of heme-oxygenase-derived CO versus NO-synthase-derived NO in hepatic ischemia-reperfusion and LPS-induced microvascular dysfunction. Interestingly, heme-oxygenase isoforms (constitutive and inducible) also exhibit differential distribution and physiological roles; CO generated by heme-oxygenase appears to control hepatic bile canniculus function via cytochrome P45-mediated Ca^{2+} regulatory mechanisms. Thus, recent findings and exciting technological approaches, as well as new gene-targeted mouse models, allow assessment of the complex and dynamic mechanisms underlying shock-induced microvascular failure."

New Aspects of NO in Shock
Speakers: Warren M. Zapol, MD; Paul L. Huang, MD, PhD; David J. Lefer, PhD; Ingo Marzi, MD; Didier Payen, MD, PhD

"The discovery of nitric oxide as a biological signalling molecule synthesized by several types of mammalian cells revolutionized contemporary understanding of various facets of medicine, including circulatory shock and its allied components. While nitric oxide synthesis is increased in some cell types as part of the response to bacterial pathogens and resulting inflammatory responses syndromes, other cell types develop impaired ability to produce nitric oxide. This polarity of response has hindered full understanding of the beneficial/detrimental profile of nitric oxide synthase isoforms in sepsis. Early studies prompted the suggestion that inhibitors of nitric oxide synthase would prove helpful as therapeutic agents in septicemic patients, whereas recent clinical and experimental studies have shown just the opposite. This symposium addresses some of the newly emerging concepts about the multifaceted aspects of nitric oxide and its role as a physiologic compensatory factor versus a pathophysiologic progenitor."

Cell Adhesion Molecules
Speakers: Allan M. Lefer, PhD; Nicholas B. Vedder, MD; Robert Rothlein, PhD; Sussan Nourshargh, PhD; Heinz Redl, PhD

"Inflammatory cells such as neutrophils, monocytes and lymphocytes are activated during shock, trauma and sepsis and accumulate in the vasculature of various organs. After the initial adherence to the vascular endothelium, leukocytes extravasate and migrate into the surrounding tissue. This inflammatory response can be beneficial in host-defense and/or cause tissue damage. In the last 10-15 years, enormous progress has been made in our understanding of the molecular mechanisms involved in leukocyte localisation at inflammatory sites. Cellular adhesion molecules (CAMs) proved to be critical for leukocyte mobilisation. Members of the selectin family are responsible for the initial contact of leukocytes with the vessel wall (rolling phenomenon). Subsequent upregulation of members of the $Beta_2$-integrin family on leukocytes as well as several members of the immunoglobulin gene superfamily are responsible for the firm adherence to endothelial cells, transmigration and possible attack on target cells. Speakers in this symposium will discuss the importance of individual CAMs in trauma, shock, sepsis and inflammation. A better understanding of the role of CAMs in these pathophysiological processes may identify new strategies for therapeutic interventions in trauma, shock and sepsis that limit inflammatory tissue injury without compromising the vital host-defense function of leukocytes."

Blood Substitutes in Shock
Speakers: Flavio Maciel, MD,PhD; Raul Coimbra, MD, PhD; George C. Kramer, PhD; Bruce Pearce, PhD

"An alternative for blood has been of utmost priority for many clinical and experimental scientists because of its antigenicity, availability (limited by donation and storage), and adulteration (chiefly viral). The major thrusts in the area for the last 30 years have been modified hemoglobin solutions and fluorocarbons. These have attractive features but also some adverse effects which have retarded their clinical use. Relatively recent clinical trials of these agents which will be discussed. These have, however, not duplicated the remarkable biological properties of blood. Colloid and crystalloid solutions are popular but lack the oxygen carrying ability of hemoglobin. Other alternative treatments for shock involve agents that reverse or block the pathophysiology involved, e.g., (naloxone, or work on a physiological basis), i.e., (hypertonic saline). The low volumes required make these agents attractive because of their portability."

Resuscitation of the Brain in Shock
Speakers: Narikyuki Hayashi, MD,Dsc; Hiromaru Ogata, MD,PhD; Kiichiro Taga, MD; Jeffrey R. Kirsch, MD; Kevin S. Lee, PhD

"Resuscitation of the brain is an important principle for the treatment of shock, since brain is the most susceptible organ to hypoxia in the body and central nervous system dysfunction is often associated with increased mortality of shock. A new approach to resuscitation of brain is based on elucidating the mechanism of brain injury during shock, including changes in abnormalities of cerebral blood flow regulation, alterations of amino acid composition and receptors, disturbances of blood-brain barrier function, and central nervous system effect of cytokine to free radicals, etc. The symposium will be focused on the recent advances in brain resuscitation and delivered by experts."

Cell Regulation and Cell Signalling
Speakers: Marilyn J. Woolkalis, PhD; James A. Cook, PhD; Mohammed M. Sayeed, PhD; Timothy G. Buchman, MD,PhD; Kazuhiro Nagata, PhD

"The last decade has witnessed an enormous growth in our understanding of the signaling pathways used by cells to regulate gene expression in response to various perturbations, such as environmental stresses, changes in hormonal milieu, exposure to microbial products (e.g., lipopolysaccharide) or various cell-cell interactions. These signaling mechanisms are important in the host's compensatory responses to infection or hemorrhage or tissue ischemia, but also undoubtedly play a crucial role in the pathogenesis of cellular dysfunction and organ injury secondary to shock or sepsis. This symposium focuses on some recent important developments in signal transduction biology particularly related to shock and/or overwhelming infection."

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Kathleen McDonough, PhD, 1996-1998
Carol L. Wells, PhD, 1998-2000

MEETINGS

National Meetings

1st June 1-3, 1978, Airlie, Virginia
William Schumer, MD, Chair
Abstracts: Circulatory Shock 5:2, 182-232, 1978
Papers: Advances in Shock Research, Vols. 1 & 2, 1979, and Metabolic and Cardiac Alterations in Shock and Trauma. Circulatory Shock Supplement 1, 1979

2nd June 7-9, 1979, Williamsburg, Virginia
David G. Reynolds, PhD, Chair
Abstracts: Circulatory Shock 6:2, 165-198, 1979
Papers: Advances in Shock Research, Vols. 3 & 4, 1980

3rd June 11-13, 1980, Lake of the Ozarks, Missouri
Lerner B. Hinshaw, PhD, Chair
Abstracts: Circulatory Shock 7:2, 187-223, 1980
Papers: Advances in Shock Research, Vols. 5 & 6, 1981

4th June 4-6, 1981, Marco Island, Florida
Sherwood M. Reichard, PhD, Chair
Abstracts: Circulatory Shock 8:2, 1981
Papers: Advances in Shock Research, Vols. 7 & 8, 1982

5th June 9-11, 1982, Smuggler's Notch, Vermont
Robert R. Wolfe, PhD, Chair
Abstracts: Circulatory Shock 9:2, 1982
Papers: Advances in Shock Research, Vols. 9 & 10, 1983

6th June 6-8, 1983, Grand Teton National Park, Wyoming
Robert W. Phillips, PhD, Chair
Abstracts: Circulatory Shock 10:3, 1983

7th June 4-6, 1984, Toronto, Canada
Glen A. Taylor, MD, Chair
Abstracts: Circulatory Shock 13:1, 1984

8th June 9-12, 1985, Baltimore, Maryland
Daniel L. Traber, PhD, Chair
Abstracts: Circulatory Shock 16:1, 1985

9th June 8-11, 1986, Scottsdale, Arizona
Gerald S. Moss, MD, Chair
Abstracts: Circulatory Shock 18:4, 1986

10th June 7-11, 1987, Montreal, Canada
Robert F. Bond, PhD, Chair
Abstracts: Circulatory Shock 21:4, 1987

11th June 5-8, 1988, Lake Geneva, Wisconsin
John C. Passmore, PhD, Chair
Abstracts: Circulatory Shock 24:4, 1988

12th June 9-12, 1989, Marco Island, Florida
Irshad H. Chaudry, PhD, Chair
Abstracts: Circulatory Shock 27:4, 1989

13th June 8-11, 1990, Durango, Colorado
H. Richard Adams, DVM, PhD, Chair
Abstracts: Circulatory Shock 31:1, 1990

14th June 2-6, 1991, Vienna, Austria
John W. Holaday, PhD, Chair
Abstracts: Circulatory Shock 34:1, 1991

15th June 7-10, 1992, Point Clear, Alabama
Donald E. Fry, MD, Chair
Abstracts: Circulatory Shock 37:1, 1992

16th June 13-16, 1993, Santa Fe, New Mexico
James A. Cook, PhD, Chair
Abstracts: Circulatory Shock Supplement 2, 1993

17th June 5-8, 1994, Big Sky, Montana
Mitchell P. Fink, MD, Chair
Abstracts: SHOCK Supplement 1, 1994

18th June 11-14, 1995, Asheville, North Carolina
Mohammed M. Sayeed, PhD, Chair
Abstracts: SHOCK Supplement 2, 1995

19th June 2-5, 1996, Grand Traverse, Michigan
James W. Holcroft, MD, Chair
Abstracts: SHOCK Supplement 2, 1996

20th June 15-18, 1997, Indian Wells, California
Edwin A. Deitch, MD, Chair
Abstracts: SHOCK Supplement 2, 1997

21st June 14-17, 1998, San Antonio, Texas
Mark G. Clemens, PhD, Chair
Abstracts: SHOCK Supplement 1, 1998

22nd June 12-16, 1999, Philadelphia, Pennsylvania
Allan M. Lefer, PhD, Chair
Abstracts: SHOCK Supplement 1, 1999

International Congresses

1st June 7-11, 1987, Montreal, Canada
Robert F. Bond, PhD, Chair
Abstracts: Circulatory Shock 21:4, 1987

2nd June 2-6, 1991, Vienna, Austria
Gunther Schlag, MD, Chair
Abstracts: Circulatory Shock 34:1, 1991

3rd Third International Shock Congress
Kazuo Okada, MD, Chair
Act City Hamamatsu, Japan
October 21-23, 1995
Abstracts: SHOCK Supplement 3, 1995

4th Fourth International Shock Congress
Allan M. Lefer, PhD, Chair
June 12-16, 1999
Abstracts: SHOCK Supplement 1, 1999

International Symposia

July 17-24, 1980, Budapest, Hungary
Cosponsors: Shock Society and International Congress of Physiology, Arisztid G.B. Kovách, John J. Spitzer, and H. B. Stoner, Chairs, Papers: Advances in Physiological Sciences, Vol. 26, Homeostasis in Injury and Shock, Pergamon Press, 1981

September 5-8, 1984, Manchester, England
"The Scientific Basis of the Care of the Critically Ill"
M.H. Irving and R.A. Little, Chairs
Partially supported by the Shock Society

FUTURE MEETINGS

23rd Annual Conference on Shock
Snowbird Ski and Summer Resort
Snowbird, Utah
June 3-6, 2000

24th Annual Conference on Shock
Marco Island Marriott Resort and Golf Club
Marco Island, Florida
June 10-13, 2001

25th Annual Conference on Shock
Big Sky Ski and Summer Resort
Big Sky, Montana
June 2-5, 2002 (tentative)

INTERNATIONAL MEETINGS

Seventh Vienna Shock Forum
Vienna, Austria
November 13-16, 1999

The 6th Indonesian-International Symposium
on Shock and Critical Care
Yogyakarta, Indonesia
November 25-28, 1999

7th International Cytokine Conference
Hyatt Regency Hilton Head
Hilton Head, South Carolina
December 5-9, 1999

Fourth International Shock Congress
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Philadelphia, Pennsylvania
June 12 - 16, 1999

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SHOCK SOCIETY
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PHILADELPHIA, PENNSYLVANIA
June 12-16, 1999

SATURDAY, JUNE 12, 1999

9:00 - 2:00 PM
Seminar A
COUNCIL MEETING

1:00 - 6:00 PM
Wyndham Foyer
Registration

6:00 - 8:30 PM
OPENING RECEPTION AT THE FRANKLIN INSTITUTE

SUNDAY, JUNE 13, 1999

7:00 - 8:00 AM
Philadelphia Ballroom
EDITORIAL BOARD BREAKFAST

7:00 - 8:00 AM
Conference Center Ballroom
Continental Breakfast

8:00 - 9:00 AM
Conference Center Hall
POSTER SESSION I, Papers 1-72

Burn/Trauma, Papers 1-20
Neutrophils, Papers 21-35
Myocardial Function, Papers 36-44
Microcirculation, Papers 45-53
Pharmacologic Agents, Papers 54-63
Pulmonary, Papers 64-72

9:00 - 9:30 AM
Wyndham D
OPENING CEREMONY

Introductions: **Allan M. Lefer, PhD**, President, Fourth International Shock Congress

The Honorable Edward G. Rendell, Mayor, City of Philadelphia

Kazuo Okada, MD,PhD, President, International Federation of Shock Societies and President Third International Shock Congress

Mohammed M. Sayeed, PhD, President, Shock Society

R.J.A. Goris, MD, President, European Shock Society

Sunday Continued

Professor M. Onda, President, Japan Shock Society

Ke-seng Zhao, MD, President, China Shock Society

Iqbal Mustafa, MD, President, Indonesian Shock Society

Saroj Sanan, PhD, President, Indian Shock Society

9:30 AM - 12:00 PM
Wyndham D

PLENARY LECTURES

9:30 am

The Evaluation and Future Trends in the Shock Patient From the Home to the Hospital
Jean-Louis Vincent, MD,PhD, The University of Brussels, Belgium
Introduced by: Gus J. Slotman, MD, UMDNJ-Robert Wood Johnson Medical School, Camden, New Jersey

10:05 am

Gender Dimorphism and the Effects of Sex Steroids in the Divergent Response to Shock
Irshad H. Chaudry, PhD, Brown University School of Medicine and Rhode Island Hospital, Providence, Rhode Island
Introduced by: John T. Flynn, PhD, Thomas Jefferson University, Philadelphia, Pennsylvania

10:40 am

Immunological Trends in the Treatment of the Shock Patient
Eugen Faist, MD, Ludwig-Maximilians-Universität, Munich, Germany
Introduced by: Edmund Neugebauer, PhD, University of Cologne, Germany

11:15 am

The History of the International Federation of Shock Societies
Kazuo Okada, MD,PhD, Teikyo University, Tokyo, Japan
Introduced by: Minoru Okuda, MD,PhD, Kyorin University, Tokyo, Japan

12:00 - 1:30 PM
Wyndham C

Buffet Lunch

1:30 - 4:00 PM
Conference Center Ballroom

SYMPOSIUM I: Cytokines in Shock
Chairpersons: **Leona Rubin, PhD**, University of Missouri, Columbia, Missouri and **Hiroyuki Hirasawa, MD**, Chiba University School of Medicine, Chiba, Japan

It has been claimed that proinflammatory cytokines play major roles in the pathogenesis of various types of shock and multiple organ dysfunction syndrome following shock. Many immunological approaches to modulate those cytokines such as monoclonal antibodies, receptor antagonists and soluble receptors, have been tried experimentally but are proven to be ineffective in clinical settings. However, we now have new therapeutic tools for the modulation of cytokines such as biological control using anti-inflammatory cytokines and continuous blood purification. In this symposium, we have two papers on the role of cytokines in shock, sepsis and injury and two papers dealing with biological control again cytokine network using interleukin-10 and bactericidal/permeability-increasing protein.

Sunday Continued

1:30 pm	**Opening Remarks** Leona Rubin, PhD and Hiroyuki Hirasawa, MD
1:35 pm	**Pro- and Anti-Inflammatory Cytokines Following Injury and Sepsis** Wolfgang K. Ertel, MD, Klinik fur Unfallchirurgie, Zurich, Switzerland
2:05 pm	**IL-10: A Counter-Regulatory Cytokine in Septic Shock** Lyle L. Moldawer, PhD, University of Florida College of Medicine, Gainesville, Florida
2:35 pm	**Differences in Chemokine and Cytokine Generation and Function** Judy A. Spitzer, PhD, Louisiana State University Medical Center, New Orleans, Louisiana
3:05 pm	**BPI: Clinical Success?** Brett P. Giroir, MD, University of Texas Southwestern Medical School, Dallas, Texas
3:35 pm	**Panel Discussion**

6:00 - 7:00 PM **Philadelphia Ballroom**	**Reception**
7:00 - 9:30 PM **Conference Center Ballroom**	**Dinner/Speaker** **PRESIDENTIAL ADDRESS: Neither Fish Nor Fowl - Collaboration and the Surgical Investigator** Edwin Deitch, MD, President-Elect, Shock Society UMDNJ-New Jersey Medical School, Newark, New Jersey

MONDAY, JUNE 14, 1999

6:30 AM	**Seventeenth Annual Presidential Run**
7:00 - 8:00 AM **Conference Center Ballroom**	**Continental Breakfast**
8:00 - 9:00 AM **Conference Center Hall**	**POSTER SESSION II**, Papers 73-145 Cytokines, Papers 73-95 Cell Signaling, Papers 96-111 Animal Models, Papers 112-122 Clinical Applications, Papers 123-133 Inflammation, Papers 134-141 Eicosanoids/PAF, Papers 142-145
9:00 AM - 12:00 PM **Wyndham C**	**SYMPOSIUM II: Endotoxin Tolerance** Chairpersons: **Ulf Haglund, MD**, Uppsala University Hospital, Uppsala, Sweden and **David Reynolds, PhD**, Iowa City, Iowa

Gram Negative sepsis and septic shock are common causes of critical illness among patients treated in the intensive care units. Despite improved therapeutic modalities based on better understanding of the underlying processes mortality in septic conditions remains very high. New approaches are therefore urgently needed to achieve better treatment results. Endotoxin is considered an important factor in the pathophysiology of Gram negative sepsis: both as a triggering factor during the initiating moments but also during the continued progression of the disease that is common in severe cases. Resistance to endotoxin has been demonstrated both as a constitutive and as an inducible phenomenon. Further understanding of

the underlying mechanisms of endotoxin resistance should provide a basis for new modalities of treatment of Gram negative sepsis.

9:00 am	**Opening Remarks** Ulf Haglund, MD and David Reynolds, PhD
9:05 am	**Overview of the Concept of Endotoxin Tolerance** Jean-Marc Cavaillon, Dr.Sc., Institut Pasteur, Paris, France
9:35 am	**Molecular Mechanisms of LPS Tolerance** H.W. Löms Ziegler-Heitbrock, MD, University of Munich, Munich, Germany
10:05 am	**Protein Kinase Alterations in Macrophage Endotoxin Tolerance** Michael A. West, MD,PhD, Hennepin Medical Center/University of Minnesota, Minneapolis, Minnesota
10:35 am	**Cross-Tolerance Between Endotoxin and Ethanol** John J. Spitzer, MD, Louisiana State University Medical Center, New Orleans, Louisiana
11:05 am	**Alcohol Causes Tolerance and Sensitization to Kupffer Cells via Mechanisms Involving Endotoxin** Ronald G. Thurman, PhD, University of North Carolina, Chapel Hill, North Carolina
11:35 am	**Panel Discussion**

9:00 AM - 12:00 PM
Wyndham A

SYMPOSIUM III: The Microcirculation in Shock
Chairpersons: **Jureta Horton, PhD**, University of Texas Southwestern Medical School, Dallas, Texas and **Janet Parker, PhD**, Texas A & M University, College Station, Texas

The microcirculation has been well documented to be a target site for pathophysiological actions of endotoxin and sepsis. Endothelial activation and leukocyte adhesion have been implicated as critical steps in microvascular injury, leading to increased permeability; thrombosis; and interactions with macrophages, platelets, acute-phase responses, hematopoietic systems, and a multitude of mediators (eicosanoids, nitric oxide, cytokines, chemokines, adhesion molecules, oxyradicals, platelet activating factor, endothelin, heme oxygenase, etc.). Recent evidence indicates leukocyte-endothelial interactions and upregulation of surface adhesion molecules not only precede leukocyte infiltration, but that this process is accompanied by oxygen free radical production and parenchymal cell death. New pharmacologic interventions (heparinase III; serine protease inhibitors; trimethylsphingosine; $ONOO^-$) decrease expression of these adhesion molecules and reduce leukocyte rolling, adherence and transmigration produced by NOS inhibition and ischemia-reperfusion. NOS blockade also enhances LPS-induced hepatic microvascular failure and leukocyte accumulation; furthermore, post-ischemic hepatic microvascular changes correlate with hepatic dysfunction. Exciting new evidence involving characteristic gene expression patterns and differential roles of heme-oxygenase-derived CO versus NO-synthase-derived NO in hepatic ischemia-reperfusion and LPS-induced microvascular dysfunction. Interestingly, heme-oxygenase isoforms (constitutive and inducible) also exhibit differential distribution and physiological roles; CO generated by heme-oxygenase appears to control hepatic bile canniculus function via cytochrome P45-mediated Ca^{2+} regulatory mechanisms. Thus, recent findings and exciting technological approaches, as well as new gene-targeted mouse models, allow assessment of the complex and dynamic mechanisms underlying shock-induced microvascular failure.

9:00 am	**Opening Remarks** Jureta Horton, PhD and Janet Parker, PhD
9:05 am	**Humoral Cell Activation in the Microcirculation During Shock** Geert Schmid-Schönbein, PhD, University of California, San Diego, California
9:35 am	**Leukocyte Rolling and Adherence in the Microcirculation** Rosario Scalia, MD,PhD, University of Catania, Catania, Italy
10:05 am	**Regulation of Leukocyte Infiltration** Konrad Messmer, MD, Institute for Surgical Research, Munich, Germany

Monday Continued

10:35 am	**The Hepatic Microcirculation in Shock** Mark G. Clemens, PhD, University of North Carolina, Charlotte, North Carolina
11:05 am	**Aberrant Heme Catabolism and Heme Oxygenase-Mediated Regulation of Liver Function in Endotoxemic Rats** Makoto Suematsu, MD,PhD, Keio University, Tokyo, Japan
11:35 am	**Panel Discussion**
12:00 - 1:00 PM	**Lunch Break**
1:00 - 2:30 PM **Wyndham C**	**YOUNG INVESTIGATOR AWARD SESSION**, Papers 146-149 Presiding: **R. Neal Garrison, MD**, University of Louisville School of Medicine, Louisville, Kentucky, Chair, Award and Honors Committee
1:00 pm	**Endotoxin Desensitization Induces Rapid Downregulation of CD14 Receptor Coupled Early Signal Transduction Events**, Paper 146 Marcella Ferlito, PhD Medical University of South Carolina, Charleston, South Carolina
1:20 pm	**Glucose-Induced, Adenosine-Mediated Portal Nitric Oxide Production is Impaired after Resuscitation from Hemorrhage**, Paper 147 Paul J. Matheson, PhD University of Louisville, Louisville, Kentucky
1:40 pm	**Interactions of Calcium-Calmodulin Dependent Protein Kinases (CaMK) and Mitogen-Activated Protein Kinases (MAPK) in Monocyte Adherence and TNF Production**, Paper 148 Matthew R. Rosengart, MD University of Washington, Seattle, Washington
2:00 pm	**Inhibition of P38 MAPK Attenuates Immunosuppression and Improves Survival in Polymicrobial Sepsis**, Paper 149 Grace Y. Song, BA Brown University School of Medicine, Providence, Rhode Island
2:30 - 3:30 PM **Wyndham A**	**DEBATE I: Is Peroxynitrite an Important Mediator of Shock?** Moderator: **Michael M. Krausz, MD**, Rambam Medical Center, Haifa, Israel Pro: **Csaba Szabo, MD,PhD**, Children's Hospital Medical Center, Cincinnati, Ohio Con: **Paul Kubes, PhD**, University of Calgary, Calgary, Canada
2:30 - 3:30 PM **Wyndham C**	**Debate II: Are SIRS and MODS Important Entities in the Clinical Evaluation of Patients?** Moderator: **Donald E. Fry, MD**, University of New Mexico School of Medicine, Albuquerque, New Mexico Pro: **John C. Marshall, MD**, The Toronto General Hospital, Toronto, Canada Con: **Arthur E. Baue, MD**, Saint Louis University Medical Center, St. Louis, Missouri

Monday Continued

3:30 - 4:30 PM **BUSINESS MEETING**
Wyndham A

FREE EVENING

TUESDAY, JUNE 15, 1999

7:00 - 8:00 AM **Continental Breakfast**
Conference Center Ballroom

8:00 - 9:00 AM **POSTER SESSION III**, Papers 150-222, 296
Conference Center Hall

Endotoxin/Sepsis, Papers 150-186
Nitric Oxide, Papers 187, 206, 296
Cellular/Molecular, Papers 207-216
Monocytes/Macrophages, Papers 217-222

9:00 AM - 12:00 PM **SYMPOSIUM IV: New Aspects of NO in Shock**
Wyndham A
Chairpersons: **H. Richard Adams**, DVM,PhD, Texas A & M University, College Station, Texas and **Eva Haglind, MD,PhD**, University of Goteborg, Goteborg, Sweden

The discovery of nitric oxide as a biological signalling molecule synthesized by several types of mammalian cells revolutionized contemporary understanding of various facets of medicine, including circulatory shock and its allied components. While nitric oxide synthesis is increased in some cell types as part of the response to bacterial pathogens and resulting inflammatory responses syndromes, other cell types develop impaired ability to produce nitric oxide. This polarity of response has hindered full understanding of the beneficial/detrimental profile of nitric oxide synthase isoforms in sepsis. Early studies prompted the suggestion that inhibitors of nitric oxide synthase would prove helpful as therapeutic agents in septicemic patients, whereas recent clinical and experimental studies have shown just the opposite. This symposium addresses some of the newly emerging concepts about the multifaceted aspects of nitric oxide and its role as a physiologic compensatory factor versus a pathophysiologic progenitor.

9:00 am **Opening Remarks**
H. Richard Adams, DVM,PhD and Eva Haglind, MD,PhD

9:05 am **Inhaled Low Dose NO in Pulmonary Disorders**
Warren M. Zapol, MD, Massachusetts General Hospital, Boston, Massachusetts

9:35 am **Development and Characterization in NOS Knockout Mice**
Paul L. Huang, MD, PhD, Massachusetts General Hospital, Charlestown, Massachusetts

10:05 am **Leukocyte-Endothelial Cell Interactions in NOS Deficient Mice**
David J. Lefer, PhD, Louisiana State University, Shreveport, Louisiana

10:35 am **Inflammatory Response of the Hepatic Microcirculation after Shock**
Ingo Marzi, MD, University of Saarland, Homburg, Germany

11:05 am **NO in Severe Sepsis**
Didier Payen, MD, PhD, Lariboisière University Hospital, Paris, France

11:35 am **Panel Discussion**

Tuesday Continued

12:00 - 1:30 PM **Buffet Lunch**
Conference Center Ballroom

1:30 - 4:15 PM **SYMPOSIUM V: Cell Adhesion Molecules**
Wyndham A Chairpersons: **Hartmut Jaeschke, PhD**, Pharmacia & Upjohn, Kalamazoo, Michigan and **Haim Bitterman, MD**, Lady Davis Carmel Hospital, Haifa, Israel

Inflammatory cells such as neutrophils, monocytes and lymphocytes are activated during shock, trauma and sepsis and accumulate in the vasculature of various organs. After the initial adherence to the vascular endothelium, leukocytes extravasate and migrate into the surrounding tissue. This inflammatory response can be beneficial in host-defense and/or cause tissue damage. In the last 10-15 years, enormous progress has been made in our understanding of the molecular mechanisms involved in leukocyte localisation at inflammatory sites. Cellular adhesion molecules (CAMs) proved to be critical for leukocyte mobilisation. Members of the selectin family are responsible for the initial contact of leukocytes with the vessel wall (rolling phenomenon). Subsequent upregulation of members of the β_2-integrin family on leukocytes as well as several members of the immunoglobulin gene superfamily are responsible for the firm adherence to endothelial cells, transmigration and possible attack on target cells. Speakers in this symposium will discuss the importance of individual CAMs in trauma, shock, sepsis and inflammation. A better understanding of the role of CAMs in these pathophysiological processes may identify new strategies for therapeutic interventions in trauma, shock and sepsis that limit inflammatory tissue injury without compromising the vital host-defense function of leukocytes.

1:30 pm **P-Selectin as a Mediator of Shock and Trauma**
Allan M. Lefer, PhD, Thomas Jefferson University, Philadelphia, Pennsylvania

2:00 pm **Modulation of CD18-Mediated Injury in Hemorrhagic Shock**
Nicholas B. Vedder, MD, University of Washington, Seattle, Washington

2:30 pm **Role of ICAM-1 in Inflammation**
Robert Rothlein, PhD, Boehringer-Ingelheim, Ridgefield, Connecticut

3:00 pm **Role of PECAM-1 in Leukocyte Accumulation**
Sussan Nourshargh, PhD, Imperial College School of Medicine, London, United Kingdom

3:30 pm **CAMs in Trauma and Endotoxemia**
Heinz Redl, PhD, Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

4:00 pm **Panel Discussion**

1:30 - 4:15 PM **SYMPOSIUM VI: Blood Substitutes in Shock**
Philadelphia Ballroom Chairpersons: **J. Ray Fletcher, MD,PhD**, University of Alabama, Mobile, Alabama and **Nelson Gurll, MD**, University of Iowa College of Medicine, Iowa City, Iowa

An alternative for blood has been of utmost priority for many clinical and experimental scientists because of its antigenicity, availability (limited by donation and storage), and adulteration (chiefly viral). The major thrusts in the area for the last 30 years have been modified hemoglobin solutions and fluorocarbons. These have attractive features but also some adverse effects which have retarded their clinical use. Relatively recent clinical trials of these agents which will be discussed. These have, however, not duplicated the remarkable biological properties of blood. Colloid and crystalloid solutions are popular but lack the oxygen carrying ability of hemoglobin. Other alternative treatments for shock involve agents that reverse or block the pathophysiology involved, e.g., (naloxone, or work on a physiological basis), i.e., (hypertonic saline). The low volumes required make these agents attractive because of their portability.

Tuesday Continued

1:30 pm	**Overview** Mauricio Rocha-e-Silva, MD,PhD, Instituto do Coracao, Sao Paulo, Brazil
2:00 pm	**Oxygen Derived Variables with Hypertonic Saline in Endotoxin Shock** Flavio Maciel, MD,PhD, University of Sao Paulo, Sao Paulo, Brazil
2:30 pm	**Immunologic Effects of Hypertonic Saline** Raul Coimbra, MD, PhD, Santa Casa School of Medicine, Santa Casa, Brazil
3:00 pm	**Blood Substitutes as Volume Expanders** George C. Kramer, PhD, University of Texas Medical Branch, Galveston, Texas
3:30 pm	**Genetic Modification of Hemoglobin in Resuscitation** Bruce Pearce, PhD, BIOPURE Corporation, Cambridge, Massachusetts
4:00 pm	**Panel Discussion**
4:30 - 5:30 PM **Wyndham A**	**DEBATE III: Is Translocation of Bacteria Important in Clinical Sepsis?** Moderator: **Thomas Vargish, MD**, University of Chicago, Chicago, Illinois Pro: **Carol L. Wells, PhD**, University of Minnesota, Minneapolis, Minnesota Con: **Robert E. Condon, MD**, Medical College of Wisconsin, Milwaukee, Wisconsin
6:30 - 7:30 PM **Wyndham CD**	**Reception**
7:30 - 9:30 PM **Wyndham D**	**Dinner/Speaker** **Industry and Academia in the New Millennium: Capturing the Scientific Promise** Thomas M. Glenn, PhD, TMG Consulting, Mobile, Alabama

WEDNESDAY, JUNE 16, 1999

7:00 - 8:00 AM **Conference Center Ballroom**	**Continental Breakfast**
8:00 - 9:00 AM **Conference Center Hall**	**POSTER SESSION IV**, Papers 223-295 Hemorrhagic Shock, Papers 223-275 Immunomodulation, Papers 276-287 Adhesion Molecules, Papers 288-295
9:00 AM - 12:00 PM **Wyndham B**	**SYMPOSIUM VII: Resuscitation of the Brain in Shock** Chairpersons: **Ke-seng Zhao, MD**, First Military Medical University, Guangzhou, China and **John W. Holaday, PhD**, EntreMed, Rockville, Maryland

Resuscitation of the brain is an important principle for the treatment of shock, since brain is the most susceptible organ to hypoxia in the body and central nervous system dysfunction is often associated with increased mortality of shock. A new approach to resuscitation of brain is based on elucidating the mechanism of brain injury during shock, including changes in abnormalities of cerebral blood flow regulation, alterations of amino acid composition and receptors, disturbances of blood-brain barrier function, and central nervous system effect of cytokine to free radicals, etc. The symposium will be focused on the recent advances in brain resuscitation and delivered by experts.

Wednesday Continued

9:00 am	**Opening Remarks** Ke-seng Zhao, MD and John W. Holaday, PhD
9:05 am	**Resuscitation of Shocked Brain by Brain Hypothermia with Replacement of Cerebral Dopamine and Pituitary Activation** Narikyuki Hayashi, MD,Dsc, Nihon University, Tokyo, Japan
9:35 am	**Effect of Hypertonic Saline on Delayed Neuronal Death in the Hippocampus CA1 Following Ischemia/Reperfusion** Hiromaru Ogata, MD,PhD, Tokai University, Tokyo, Japan
10:05 am	**Brain Resuscitation and NMDA Receptors from the Point of View of Ischemic Tolerance** Kiichiro Taga, MD, Niigata University School of Medicine, Niigata, Japan
10:35 am	**Mechanism of Injury in Cerebral Ischemia** Jeffrey R. Kirsch, MD, Johns Hopkins University, Baltimore, Maryland
11:05 am	**Modulation of Proteolysis in Neuronal Recovery** Kevin S. Lee, PhD, University of Virginia, Charlottesville, Virginia
11:35 am	**Panel Discussion**

9:00 AM - 12:00 PM
Wyndham A

SYMPOSIUM VIII: Cell Regulation and Cell Signalling
Chairpersons: **Ronald V. Maier, MD**, University of Washington, Seattle, Washington and **Mitchell P. Fink, MD**, Beth Israel Hospital, Boston, Massachusetts

The last decade has witnessed an enormous growth in our understanding of the signaling pathways used by cells to regulate gene expression in response to various perturbations, such as environmental stresses, changes in hormonal milieu, exposure to microbial products (e.g., lipopolysaccharide) or various cell-cell interactions. These signaling mechanisms are important in the host's compensatory responses to infection or hemorrhage or tissue ischemia, but also undoubtedly play a crucial role in the pathogenesis of cellular dysfunction and organ injury secondary to shock or sepsis. This symposium focuses on some recent important developments in signal transduction biology particularly related to shock and/or overwhelming infection.

9:00 am	**Opening Remarks** Ronald V. Maier, MD and Mitchell P. Fink, MD
9:05 am	**Receptor Regulation of MAP Kinase Function** Marilyn J. Woolkalis, PhD, Thomas Jefferson University, Philadelphia, Pennsylvania
9:35 am	**G-Protein Coupled Signalling Pathways Activated by Endotoxin and Altered by Endotoxin Tolerance** James A. Cook, PhD, Medical University of South Carolina, Charleston, South Carolina
10:05 am	**Leukocyte Signalling Mechanisms** Mohammed M. Sayeed, PhD, Loyola University Medical Center, Maywood, Illinois
10:35 am	**Degrees of Freedom** Timothy G. Buchman, MD,PhD,Washington University School of Medicine, St. Louis, Missouri
11:05 am	**Regulation and Function of collagen-Specific Molecular Chaperone HSP47** Kazuhiro Nagata, PhD, Kyoto University, Kyoto, Japan
11:35 am	**Panel Discussion**

DINNER SPEAKERS

Sunday Evening, June 13, 1999

Neither Fish Nor Fowl - Collatoration and the Surgical Investigator

Edwin A. Deitch, MD
Shock Society President-Elect
UMDNJ- New Jersey Medical School
Newark, New Jersey

Tuesday Evening, June 15, 1999

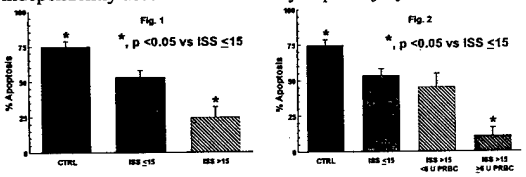
Industry and Academia in the New Millennium: Capturing the Scientific Promise

Thomas M. Glenn, PhD
TMG Consulting
Mobile, Alabama

1

BLOOD TRANSFUSION INDEPENDENTLY DELAYS NEUTROPHIL APOPTOSIS IN TRAUMA PATIENTS.
W. Biffl, E. Moore, P. Offner*, J. Gabriel*, M. Brown*, C. Silliman*. Denver Health Med Ctr, Denver, CO 80204.

Delayed neutrophil (PMN) apoptosis prolongs the functional lifespan of PMNs, potentially exacerbating PMN-mediated tissue injury leading to postinjury multiple organ failure (MOF). We previously identified blood transfusion as an independent predictor of postinjury MOF. We recently found delayed PMN apoptosis in severely injured patients receiving blood transfusions. In vitro data suggest that stored blood delays PMN apoptosis. We **hypothesized** that delayed postinjury PMN apoptosis is related to early blood transfusion. **Methods:** PMNs harvested from injured patients (n=20) six hours postinjury were isolated (buffy coat method) and incubated (5% CO_2,37°C) in RPMI 1640. Apoptosis was assessed after 24 hr using acridine orange/ethidium bromide and fluorescence microscopy. Patients were subgrouped based on ISS and early transfusion requirements. Apoptosis rates were compared among groups using Student t-test. Data are presented as mean±SEM. **Results:** PMN apoptosis appeared delayed in a "dose-dependent" manner (i.e.,related to ISS) in patients (Fig. 1). However, when adjusted for blood transfusion (≥6 units), this ISS effect was lost (Fig. 2). Multiple linear regression confirmed that transfusion, but not ISS, was independently associated with delayed postinjury PMN



apoptosis. There was a trend (p=.09) toward association between delayed apoptosis and MOF. **Conclusion:** Blood transfusion independently delays PMN apoptosis, which may be associated with postinjury MOF. Inflammatory agents contaminating stored blood likely mediate this effect.

2

INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE PREVENTS LUNG NEUTROPHIL DEPOSITION AND DAMAGE IN BURNED RATS.
J.-S. Chen*, L.-W. Chen*, C.-M. Hsu* and H.-L. Chen*, (Spon:M.-S. Liu).
Veterans General Hospital-Kaohsiung and National Sun Yat-Sen University, Kaohsiung, Taiwan.

Severe burn injury is often associated with damage to tissues distant from the injured skin. After burn, activation of neutrophils plays a role in adult respiratory distress and multiple organ failure. Recently, gut ischemia/reperfusion was shown to be the central event initiating postinjury multiple organ failure and it is suggested that NO is involved in the physiology and response to critical illness of the GI tract. In our previous studies, we demonstrated that thermal injury could induce intestinal mucosal iNOS activity. Specific inhibition of iNOS activity with SMT ameliorated the barrier function and the BT occurrence. In this study, the role of NO and effect of iNOS inhibitor on the lung neutrophil deposition and damage after burn were investigated. The SD rats were randomly divided into three groups (n = 6 each group): sham burn with saline injection (5 ml, i.p.), 35 % TBSA scald burn with saline injection (5 ml, i.p.), and 35 % TBSA scald burn with SMT injection (5 mg/kg, i.p.). Eight hours postburn, lung tissues were harvested for myeloperoxidase assay, histology study, and tissue injury quantitation. Results show that the thermal injury induced a significant 2-fold increase in MPO activity and concentration of Evans blue within the lung. Inhibition of iNOS by SMT injection significantly reduced ($p < 0.05$) the lung MPO activity and Evans blue extravasation in burned rats when compared with saline injected rats. In addition, the histology findings also support the beneficial effect of SMT on lung after thermal injury. The data suggest that NO is an important mediator for the lung damage induced by thermal injury, and SMT given immediately after burn attenuated the induced neutrophil deposition and tissue damage in lung.

3

ENDOGENOUS ADENOSINE AND SECONDARY INJURY AFTER CHEST TRAUMA
KA Davis*, TC Fabian, DN Ragsdale*, LL Trenthem*, KG Proctor. Univ. of Tennessee Health Science Center, Memphis, TN 38163

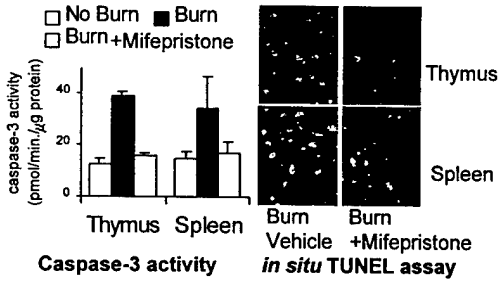
We have previously shown: 1) adenosine is an endogenous anti-inflammatory autacoid whose properties can be exploited with the pharmacologic agent, acadesine; 2) a progressive secondary injury in the contralateral (uninjured) lung after unilateral blunt chest trauma, is due (in part) to activation and sequestration of leukocytes (WBC), neutrophils (PMNs) and other inflammatory cells. Thus, we hypothesized that acadesine would ameliorate secondary injury after chest trauma. **METHODS:** Cross bred pigs (n=21) received a right sided pulmonary contusion, using methods previously described. Either acadesine (1 mg/kg bolus + 0.2 mg/kg/min) or saline vehicle were administered 15 min prior to injury, and were continued for 12 hr or death. Hemodynamics, pulmonary function, bronchoalveolar lavage (BAL) protein, and BAL WBCs were monitored serially. At autopsy, wet/dry weight ratios were assayed for lung edema and myeloperoxidase (MPO) was assayed for parenchymal PMN infiltration. **RESULTS:** With acadesine vs. saline, systemic hemodynamics were similar, but survival improved (9/10 vs. 4/11, $p<0.04$). Injury caused a 3-4 fold increase in BAL WBCs bilaterally, a 10-20 fold increase in BAL protein bilaterally, and a 2 fold increase in lung wet/dry ratio (all $p<0.05$). Acadesine had no effect on BAL WBCs, but BAL protein and lung wet/dry ratios were reduced 30-50% bilaterally (both $p<0.05$). Furthermore, MPO in the contralateral uninjured lung was reduced by 45% ($p<0.05$). **CONCLUSIONS**: After pulmonary contusion, increased endogenous adenosine ameliorated WBC-dependent destruction of the alveolar capillary membrane and reduced lung edema, prolonging overall survival. Therefore, adenosine-regulating agents may have therapeutic potential after chest wall trauma.
Support: Grant from ONR and scholarship from AAST

4

ORGAN APOPTOSIS IS DEPENDENT ON GLUCOCORTICOIDS BUT NOT FasL EARLY AFTER BURN INJURY.
K. Fukuzuka*, R. Minter*, J. Rectenwald*, C.K. Edwards III*, L. Moldawer, D. Mozingo. Univ. Florida, Gainesville, FL. 32610.

Although increased apoptosis in circulating lymphocytes has been reported after burn injury, little is known about apoptosis in solid organs induced by TNFα, FasL and glucocorticoids. We have observed apoptosis in thymus and spleen early (3 hours) after burn injury independent of TNFα and LPS. Methods: C57BL6 female mice (n=60) were divided into vehicle burn group, glucocorticoid receptor antagonist (mifepristone) or murine Fas fusion protein (mFasFc)

treated burn groups and unburned vehicle group. 3 hours after burn injury, histologic analysis, *in situ* TUNEL staining, and caspase-3 activity measurements were performed in thymus and spleen. Results: Mifepristone decreased apoptosis and caspase-3 activity after burn injury, whereas mFasFc reduced caspase-3 but did not affect apoptosis in burned animals. Conclusions: Corticosteroids, but not FasL induce apoptosis through increasing caspase-3 activity in thymus and spleen 3 hours after burn.



5

BENEFITS OF EARLY TRANSFUSION ON CORTICAL O_2 SUPPLY/ DEMAND DURING RESUSCITATION OF TRAUMATIC BRAIN INJURY (TBI). JB Gibson*, RA Maxwell*, JB Schweitzer*, LL Trenthem*, TC Fabian, and KG Proctor Depts. of Surgery, Pathology, and Physiology, University of Tennessee Health Science Center, Memphis TN 38163.

Background: Brief episodes of hypotension after TBI increase morbidity/mortality as much as 3-5x. The Brain Trauma Foundation has recommended patient management guidelines, but there are virtually no clinically-relevant experimental simulations to rigorously test these ideas. Therefore, we compared three fundamentally different resuscitation strategies. Methods: Anesthetized, ventilated (FiO_2=0.4) swine received TBI via fluid percussion (>5 ATM) to the pariental cortex followed by 30% arterial hemorrhage. After 1 hr, fluid resuscitation consisted of either unlimited saline (SAL, n=11) or a fixed volume of either plasma + 2x saline (PLA, n=5) or shed blood + 2x saline (BLD, n=7). Cortical O_2 supply/demand was evaluated with a sagittal sinus catheter. Results: With all three fluids, cardiac index and mixed venous O_2 saturation immediately corrected with resuscitation, and hematocrit never fell below 60% baseline. But even with 5x the shed blood volume restored, cerebral perfusion pressure (CPP, mm Hg) and sagital sinus PO_2 (SS PO_2, mm Hg) remained depressed with SAL or PLA vs BLD. In addition, 10% $FiCO_2$ unmasked further disturbances in cortical glucose uptake (Glu Δ, mg%) and autoregulation. By 72 hrs, histopathology was correlated with these changes.

@60 min	Before $FiCO_2$ challenge			During $FiCO_2$ challenge		
post	*CPP	*PO_2 ss	Glu Δ	*CPP	*PO_2 ss	*Glu Δ
SAL	53±5	31±2	14±3	41±3	48±5	11±5
PLA	81±9	28±2	7±1	72±7	57±5	5±2
BLD	91±8	38±3	9±2	69±4	67±5	-2±2

*=p≤0.05 between or within treatment groups

Conclusion: Crystalloid and colloid both restored systemic, but not brain, O_2 supply/demand, suggesting that blood transfusion thresholds after TBI may be different than after other types of severe injury.

6

DYSBALANCE OF SOLUBLE TUMOR NECROSIS FACTOR RECEPTORS P55 AND P75 AFTER MULTIPLE TRAUMA-CORRELATION TO IL-6, -10 AND 13.

T. Hensler*, S. Sauerland *, S. Heß* and E. Neugebauer. Biochem. & Exptl. Abt., Ostmerheimer Str. 200, 51109 Köln.

Different pro- and anti-inflammatory mediators are involved in the restoration of the disturbed physiological balance after accidental-trauma. It was the aim of this study to further elucidate the mediator response and its relationship to the type of injury in patients with isolated severe head trauma (SHT, Grp. I), in patients with SHT + multiple injuries (Grp. II), and in patients with multiple injuries without SHT (Grp. III). Plasma was collected from a total of 93 patients (days 1 – 3 every 6 hours, 4 – 10 daily, and days 14, 21 and 28. Mediators were analysed by ELISA and evaluated using the Spearman-correlation-coefficient (r). IL-6 elevations during the first 24 hours of accidental-trauma were closely accompanied by increases of anti-inflammatory IL-10 -levels and declined rapidlys during the next 3 days, in the absence of further clinical complications ($r = 0,66$; $p < 0,005$). There was a smaller correlation between anti-inflammatory IL-10 and sTNFr-levels (p55: $r = 0,379$; p75: $r = 0,214$; p for both $< 0,005$). The IL-6, -10 and sTNFr -levels in Grp. I were lower than the detected levels in Grp. II and III. Anti-inflammatory IL-13 -levels were not elevated after accidental- or surgical-trauma and did not correlate to IL-10 ($r = 0,046$; $p = 0.88$). In contrast there was a high correlation between both sTNFr-subtypes ($r = 0,722$; $p < 0,005$). The sTNFr p55/p75 -ratio in traumatized patients of all three groups was significantly elevated within 3 hours after trauma and returned to normal ratios after 36 hours. This posttraumatic shift to the sTNFr p55-subtype was lowest in patients of Grp. I. We concluded that monitoring of IL-6, -10 and sTNFr-levels may help in both, understanding of the clinical and immunological situation of the traumatized patient and decision making (timing, type) for secondary surgical interventions (e.g. osteosynthesis).

[Supported by BMBF (FKZ 01 KO 9517)]

7

A MURINE MODEL OF BURN TRAUMA. J. Horton, J. White*, D. Maass*, B. Sanders*, J. Murphy and B. Giroir, UT SWMC, Dallas, TX 75235-9160.

We have shown previously that severe burn trauma impairs cardiac contraction/relaxation in several animal models including rats, guinea pigs, rabbits and sheep. In addition, we have shown that burn injury over 35% TBSA in humans is associated with decreased cardiac stroke work in the early postburn course despite elevated filling pressures. The availability of transgenic and knockout mice have suggested that developing a murine burn model would allow us to examine molecular mechanisms by which a cutaneous burn produces distal organ injury. To this end, adult C57 mice (20-25g) were anesthetized with methoxyflurane supplemented with O_2 flow. A 3° burn over 40% TBSA was produced by heating 3mm brass probes in boiling water and probes were placed (5 sec) in rapid succession on the back/sides for a total of 8 applications. Lactated Ringer's (3ml/kg/%burn) was given IP; sham burns were included for controls. We first confirmed that this resuscitation regimen was sufficient to allow survival (97%) for 5 days postburn. $[Ca^{2+}]_i$ measured in sham murine cardiomyocytes (collagenase digestion, Fura-2AM loading) was similar (95±8nM) to that measured in sham rat myocytes (97±10nM). We then established that indices of cardiac function (LVP and +/- dP/dt max) measured in hearts from sham burned mice (Langendorff) (84±1mmHg, 2000±41 mmHg/sec and 1550±50 mmHg/sec) were similar to values measured

in adult rats (89±3, 2199±32, 1776±68) and guinea pigs (82±3, 1662±55, 1357±57). Compared to values measured in sham mice, burn trauma produced cardiac dysfunction as indicated by a fall ($p<0.05$) in LVP (56±4), +dP/dt max (1393±110), and –dP/dt max (1023±40). These studies confirm that burn trauma in mice produces cardiodynamic responses similar to those seen in other rodent models, providing an adequate model to assess molecular mechanisms that contribute to organ dysfunction.

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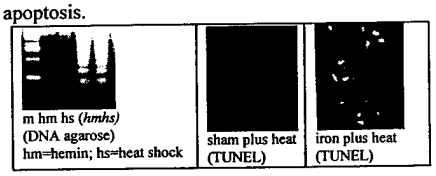
IRON-LADEN TISSUES SUSTAIN APOPTOSIS FOLLOWING PYREXIA. AK Jacobs, RS Hotchkiss, T Lin, PS Swanson, TG Buchman. Washington Univ., St. Louis, MO 63110.

Iron is both essential to and toxic to mammalian cells. Despite well-developed systems of binding proteins which recycle iron liberated from heme, iron ions can accumulate in the microenvironment of injury, generating reactive oxygen species via Fenton chemistry. Since fever commonly accompanies breakdown of injured tissues and blood, we hypothesized that such pyrexia could modulate the effect of free iron on tissue. We injected natural iron compounds (free hemoglobin or hemin) or synthetic iron complexes (ferric ion complexed to 8-hydroxyquinoline) into the subcutaneous tissues of ketamine/xylazine anesthetized ND4 mice and subsequently immersed the mice into water baths at normal (37 C) or elevated (39 C/30 min or 42 C/10 min). Within 18 hours, the iron-laden, heated tissues ulcerated while controls did not. The animals were killed. Electron microscopy, TUNEL analysis and agarose gel electrophoresis of DNA all showed apoptosis. To determine the trigger of apoptosis, the spin trap DMPO was injected into the ulcer at harvest ; ESR showed the presence of DMPO-hydroxyl adduct. To confirm the pivotal role of iron-generated excess reactive oxygen species, animals were loaded with the iron-chelator, deferroximine or the SOD mimetic, MnTBAP. These drugs blocked development of ulcers and markedly reduced the apoptosis in the subcutaneous tissues. We conclude that excess iron (bound to a synthetic or natural carrier) places tissues at risk. This risk is realized at clinically relevant pyrexial temperatures and leads to tissue dissolution by apoptosis.



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THERMAL INJURY ALTERS THE INSULIN-LIKE GROWTH FACTOR (IGF) SYSTEM. C.H. Lang and R. A. Frost. Penn State College Medicine., Department Cell. Molec. Physiology, Hershey, PA.

Previously studies in patients and experimental animals have demonstrated that circulating levels of IGF-I are markedly decreased after thermal injury. However, alterations in IGF-I content in various target tissues have not been assessed. Male Sprague-Dawley rats were deeply anesthetized and burned over ~30% of their total body surface area. Time-matched control animals were treated similarly, but not burned. 24-h after thermal injury, rats were anesthetized, and blood and selected tissues were collected. The plasma IGF-I concentration was decreased 54% ($P < 0.05$) in burned rats, compared to control values. Thermal injury decreased the IGF-I content in liver by 40% ($P < 0.05$). Since the liver is the primary site of blood-borne IGF-I this decrease in hepatic IGF-I is consistent with the drop in plasma IGF-I levels. Similarly, burn animals also demonstrated a significant decrease in IGF-I content in both the gastrocnemius (60%) and EDL (74%), compared to muscles from control rats. In contrast, the IGF-I content in kidney was not significantly different between groups. Thermal injury also altered the plasma concentration of several of the IGF binding proteins (IGFBP). Burned rats demonstrated a marked increase in IGFBP-1 and a decrease in IGFBP-3. The results of the present study indicate that burn decreases IGF-I levels in blood and skeletal muscle, and suggests that reduction in the content of this anabolic hormone in muscle may be in part responsible for the wasting observed in this catabolic condition. (Supported by NIGMS 38032).

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THE CONSEQUENCES OF MULTIPLE TRAUMA AND INJURY PATTERN ON SEVERELY HEAD INJURED PATIENTS. U. Lehmann[1*], H-C. Pape[1*], M. Winny[1], S. Zech[1], E. Rickels[2], M. Lorenz[2], Dep. Traumatol.[1] / Neurosurg.[2]; Med. Sch. Hannover

Objective: The aim of the study was to find out the influence of injury pattern, the effects of primary operative treatment and the consequences of organ failure during stay on intensive care unit (ICU). Methods: From 3/95 to 3/97 83 patients with severe head injury (AIS≥3) were prospectively investigated. Severity and pattern of injury were evaluated by injury severity score (ISS). Patients were subdevided into a group with isolated head injury (HI) and a group with head injury and multiple trauma (HI+MT; additionally AIS≥3). Outcome was determined 12 months after trauma by Glasgow Outcome Scale: GOS 1, 2+3 bad (grp. I=HI, grp. III= HI+MT) and GOS 4+5 good (grp. II=HI, grp. IV=HI/MT) outcome. Data of hemodynamics were collected and the MODS (Marshall) score was used to assess the extent of organ failure on ICU. Differences between groups were tested by ANOVA, post hoc comparisons by LSD-test. Results: ISS was significantly different between the groups ($p<0.0001$; I:29.9±9.6; II:19.9±6.7; III:40.6±8.8; IV:30.8±9.1), AIS head was significantly higher in the groups with bad outcome ($p<0.0006$: I vs II; $p<0.004$: III vs IV). Intraoperatively delivered amount of blood (2500ml) was significantly higher in group III ($p<0.05$), the mean arterial blood pressure decreased in all groups, but not significantly. Intracranial pressure (ICP; $p<0.0001$; I: 23.2±3.8; II: 21.2±2.2; III: 23.8±3.8; IV: 16.7±4.7) and cerebral perfusion pressure ($p<0.02$) were significantly different between the groups, especially ICP was significantly lower in group IV. MODS was also significantly different ($p<0.0001$) between the groups (I: 4.9±1.0; II: 3.4±0.3; III: 5.9±1.6; IV: 5.2±0.3). Conclusion: Injury pattern, perioperative hypotension and severity of organ failure are important factors of patients outcome after severe head injury.

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EFFECTS OF A NOVEL ANTI-OXIDANT FOLLOWING SEVERE BLUNT CHEST TRAUMA. RA Maxwell*, JB Gibson*, AR Swallows*, L Ma*, CJC Hsia*, TC Fabian, KG Proctor Depts of Surgery and Physiology, Univ. of Tennessee Health Science Center, Memphis TN 38163, and SynZyme Technology, Irvine CA 92618.

Background: Our previous studies have implicated activated neutrophils (PMNs) and prostaglandins in the progressive respiratory failure after severe pulmonary contusion (PC). Since reactive oxygen metabolites (ROM) are closely associated with both factors, we examined actions of a novel antioxidant on the pathogenesis of leukocyte mediated alveolar capillary disruption in a clinically-relevant model. Methods: Cross-bred, anesthetized, ventilated swine received a unilateral right PC, followed by a 30% hemorrhage. After 1 hr, an infusion (2ml/kg/hr iv x 6 hr) of either PNS+Tempol (PNS^+, N=9), 5% Dextran (D, N=6), or lactated Ringers (LR, N=13) was begun, followed after 15 min by resuscitation with LR (3x shed blood volume plus further LR as needed to maintain hemodynamics). PNS^+ was prepared by covalently labeling 5% dextran-70 with 30 mM bound nitroxide, then adding 2 mg/ml free Tempol. Hemodynamics, ABG's, CBC's, lactate, pulmonary function and bronchoalveolar lavage (BAL) were serially obtained for 6 hrs. Results: PNS^+ vs D or LR tended to reduce IVF requirement (5775±417 vs 7097±770; p=0.12 or 7196±549 ml; p=0.06), PMNs ($x10^6$/ml) and protein (mg%) in right (R) and left (L) lung BAL:

	PMN, R	Protein, R	PMN, L	Protein,L
LR	17.3±4.0*†	3687±899†	14.3±3.8	1486±357†
D	15.9±3.1†	2560±498†	10.4±2.4	1955±671*†
PNS^+	7.6±2.0	1560±350	7.6±1.8	609±153

*=p≤0.05 vs PNS^+; †=p≤0.05 vs respective baseline

Conclusions: After blunt thoracic trauma: 1) ROM elaborated from injured vascular endothelium, alveolar macrophages or infiltrating PMNs may have a role in inflammatory lung injury. 2) anti-oxidants in resuscitation fluids may have therapeutic potential for ameliorating secondary lung injury. Supported by Office of Naval Research

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CORONARY AND PULMONARY ENDOTHELIAL *IN VITRO* RESPONSE TO THERMAL INJURY.

JT Murphy, HT Wheeler,* S Duffy.* UT Southwestern Med. Center, MC#9158, Dallas, Tex. 75235-9158

Loss of vascular integrity following thermal injury (capillary leak) is thought to result from a systemic sublethal endothelial injury or "activation". We propose that post-burn endothelial activation is not a uniform change in endothelial phenotype, but is time-dependent and occurs with organ-specificity. In this study, we examine the response of two distinct endothelial cell (EC) types to serum isolated from patients with severe thermal injury. Serum was isolated from burn patients (>40%TBSA) at 4 (Early) and 24 hrs (Late) post-injury. Human coronary and pulmonary EC were exposed to 20% pooled burn and control serum for 24 hours at $37^0C/5\%CO_2$. Vasomotor effector release was measured by EIA, ICAM-1 expression by immunohistochemical staining and monolayer permeability by albumin diffusion. Coronary EC in Early serum released significantly more ET-1 (1746±681) and less PGI_2 (303±66) compared to Controls (282±81 and 558±98pg/10^6cells), while NO release was unchanged. In Late burn serum, ET-1 release diminished to baseline while NO release was significantly enhanced vs. control (170±2.7 vs. 116±5.7uM). Pulmonary EC responded to Early serum stimulation with significant reduction in ET-1 (467±1.6 vs.191±3) and increase in PGI_2 (499±78 vs. control 305±35), while NO was not effected. In Late burn serum, pulmonary EC release of ET-1 was further diminished (64±1.6), PGI_2 was reduced to Control levels and NO was enhanced (170±2.7 vs. control 116±5). Both EC lines demonstrated enhanced ICAM-1 expression in response to Early and Late burn serum. Permeability to albumin was unchanged by Early serum, but significantly enhanced by Late serum in both EC lines (coronary: 30 vs 0.8; lung: 53 vs. 1.7pg/ml). These data suggest that human endothelium isolated from different vascular beds are affected by burn injury in a unique manner with respect to time post-burn and site of origin.

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TIMING OF MAJOR SURGERY AND CYTOKINE RELEASE AFTER TRAUMA: PREDICTION OF POST-OP COMPLICATIONS ?

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Inadequate timing of major reconstructive operations in multiple trauma patients is discussed to impose an additional burden. This may aggravate the immunologic mechanisms involved in the development of organ failure (MOF). *Methods:* 128 polytrauma patients (ISS>20) were differentiated according to the timing of sec. surgery. Interleukin-6 serum levels were monitored perioperatively during and after secondary surgery. Def.: Multiple organ failure: Knaus'criteria; sec. surgery: reconstructice surgery > 3 hours duration and >24 hours post trauma. *Results:* Interleukin-6 values on admission were higher in group +MOF (891±87 pg/dl) than in group -MOF (327±57 pg/dl, p=0.02). Secondary surgery on days 2-4 was associated with a higher incidence of MOF (47%) than sec. surgery on days 6-8 (15%, p=0.01). A combination of initial Il-6 values > 500 and surgery on day 2-4 positively correlated with the development of MOF (r=0.96, p<0.001), whereas initial Il-6-values > 500 and surgery on days 6-8 did not (r=0.57, p<0.07). *Conclusion:* Both the inflammatory response, and the timing of major secondary surgery may affect the further clinical course in patients with blunt trauma. The combination of these parameters may serve as an additional tool to predict outcome.

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AQUAPORIN-1 IN AN OVINE MODEL OF BURN AND SMOKE INJURY. F. Schmalstieg*, K. Seojima*, L. Traber, H. Rudloff*, and D. Traber. The University of Texas Medical Branch, Galveston, TX 77555.

Pulmonary injury and mortality in combined burn and smoke injury is more severe than in either injury alone. A constant characteristic of this lesion is a large transvascular fluid flux that occurs both in the lung and peripheral microvasculature. OBJECTIVES: In this work we tested the hypothesis that the water channel, aquaporin-1, is involved in the transvascular fluid flux. Aquaporin-1 (AQP-1) is one of a number of recognized proteins that support water transport and is present in microvascular endothelium. METHODS: Sheep were subjected to 38 breaths of cotton smoke and/or 40% full thickness burn. Some of these sheep were treated with anti-IL-8 monoclonal antibody (K221). Messenger RNA was isolated from ovine lungs after these injuries by affinity

chromatography. The resulting mRNA was reverse transcribed to cDNA. The cDNA was quantitated using a competitive internal standard (MIMIC). RESULTS: AQP-1 was elevated in lung tissue by 8 h after injury and remained elevated through 48 h of study. The relative order of increase in AQP-1 mRNA was burn and smoke > burn > smoke > control. Furthermore, linear regression analysis between lung lymph flow and concentration of AQP-1 mRNA revealed an inverse relationship between these two variables. We previously showed that anti-IL-8 antibody (K221) decreased transvascular fluid flux in burn and smoke injured sheep. Consistent with the inverse relationship between lung lymph flow and AQP-1 mRNA, animals treated with anti-IL-8 had significantly higher concentrations of AQP-1 mRNA at 48 h post-injury. CONCLUSIONS: We conclude that AQP-1 mRNA is elevated by burn and smoke, burn, and smoke injuries. The negative relationship between lung lymph flow and AQP-1 mRNA suggests that AQP-1 expression is protective and functions in increased uptake of interstitial fluid. This behavior is likely governed by differences in oncotic pressure between the microcirculation and the interstitial space. Furthermore, the ability of anti-IL-8 to decrease fluid flux in burn and smoke injury may be the result of increased expression of AQP-1.

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BURN SERUM POTENTIATES ALVEOLAR MACROPHAGE (aMϕ) TNF-α AND IL-6 RELEASE. C. Schulman*, S. Duffy*, F. Nwariaku*, R. Turnage. Univ. of Texas Southwestern Medical Ctr, Dallas TX 75235.

Previous studies in our laboratory have demonstrated that aMϕ's from animals sustaining burn injury have an exaggerated cytokine response to endotoxin (LPS). This study tests the hypothesis that burn serum primes naïve aMϕ's to release increased amounts of TNF-α and IL-6. Furthermore, this study examines the priming effect of burn serum on two immortalized macrophage cell lines, NR8383 and RAW 264.7. Alveolar macrophages were harvested from BAL of normal uninjured rats and cultured for 24 hrs in RPMI with 20% burn (40% TBSA burn) or sham serum after the addition of LPS (0 – 10 μg). Cell culture supernatants were sampled for TNF-α and IL-6 (ELISA). The immortalized cell lines underwent identical experimental conditions. The concentration of LPS utilized in these studies was based on the linear region of a dose-response curve between [LPS] and cytokine release. The data are expressed as mean ± SEM and analyzed by ANOVA.

N > 10	Alveolar macrophages		RAW 264.7	
	[LPS] (0μg)	[LPS] (10 μg)	[LPS] (0 μg)	[LPS] (1 μg)
TNF (ng/ml)				
Sham	1.1 ± 0.1	2.4±0.2	0 ± 0	0 ± 0
Burn	2.2 ± 0.2	5.0±0.2*	16.7 ± 1.6	13.7±1.4
IL-6 (pg/ml)				
Sham	26 ± 2	156 ± 6	421 ± 8	624 ± 37
Burn	67 ± 9	241 ± 8*	415 ± 17	313 ± 22

* $p < 0.05$ vs Burn serum, 0 μg LPS & Sham serum , 10 μg LPS

Data for NR8383 cells was similar to that for the RAW 264.7 cells (data not shown). These data demonstrate that burn serum primes naïve aMϕ for increased TNF-α and IL-6 release but this priming effect is not seen in the NR8383 or RAW 264.7 immortalized cell lines.

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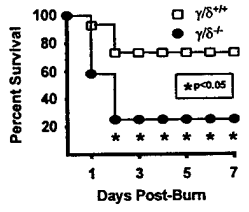
THE ROLE OF γ/δ T-CELLS IN THE IMMUNE RESPONSE TO MAJOR BURN TRAUMA. M.G. Schwacha, A. Ayala and I.H. Chaudry. Ctr. for Surgical Research, Dept. of Surgery, Brown University and Rhode Island Hospital, Providence, RI 02903.

Studies have shown that thermal injury (TI) causes suppression of cell mediated immunity. Both T-cell dysfunction and the increased production of immuno-inflammatory mediators by macrophages (Mϕ) have been implicated as causative factors. With regards to T-cells, previous studies have primarily examined the effects of TI on α/β T-cells. Although γ/δ T-cells are important in the immune response to a number of pathogens their role in the immune response to TI remains unclear. The aim of our study, therefore, was to determine the role of γ/δ T-cells in survival and the immune response following TI in C57BL/6 mice lacking γ/δ T-cells ($\gamma/\delta^{-/-}$) and matched normal mice ($\gamma/\delta^{+/+}$). The mice were subjected to a 25% TBSA 3rd degree scald burn and survival was assessed. At 7 days post-TI splenic Mϕ were isolated from surviving animals and functional activity determined. The results indicate that $\gamma/\delta^{-/-}$ mice had significantly greater mortality after TI with ~75% of the mice dying as compared to ~25% mortality in the $\gamma/\delta^{+/+}$ group (Figure). Moreover, Mϕ PGE_2, IL-6, TNF, and IL-10 production in response to LPS was significantly increased in Mϕ from $\gamma/\delta^{+/+}$ mice after TI. In contrast, IL-6 and TNF production by Mϕ from $\gamma/\delta^{-/-}$ mice subjected to TI was significantly attenuated. The increased production of anti-inflammatory mediators (PGE_2, IL-10) after TI, however, was not reduced in Mϕ from $\gamma/\delta^{-/-}$ mice. Thus, the results implicate a "dual role" for γ/δ T-cells after TI since mice lacking γ/δ T-cells were; (i) more susceptible to the lethal effects of TI early post-injury (≤ 2 days) and (ii) did not express a pro-inflammatory Mϕ phenotype (↑ IL-6, TNF) later post-injury (7 days). Therefore, selective modulation of γ/δ T-cell activity after major burn trauma may provide therapeutic advantages for such patients.



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SERUM MELATONIN LEVELS IN BURN INJURY Jane Shelby, Linda Edelman*, Shixuan Xu*, Ryan Watt*, University of Utah, Salt Lake City, UT 84132

Melatonin has antioxidant and immunomodulatory activity and is an important regulator of biologic rhythms. The aim of this study was to measure serum melatonin (MEL) production post injury, and to describe concurrent changes in cytokine response. **Methods and Results**: Male guinea pigs were given a 20% tbsa burn and postburn. daytime (10:00am) serum MEL levels assessed. In young animals (2-4 mos), there was a significant increase in MEL production 24 and 36 hours postburn ($P<0.01$), however, in older guinea pigs (5-8 mos), this increase was not observed. These results suggest that the injury event is associated with increased daytime levels of MEL in younger animals, but whether this is a disruption in circadian rhythm or overall increase in MEL production is unclear with sampling at only one daytime time point. In a second experiment, C3H/HEN mice (8 wk males) were given a 20% tbsa injury and euthanized at 1, 3, 6, 24, and 72hrs postburn. Serum was collected and assessed for MEL. Spleens from control and injured mice were harvested at 1, 3, 6, 24, and 72hrs postburn and supernatants assayed for cytokines by ELISA., Similar to the results in the guinea pig experiment, injured mice showed an increase in daytime MEL, reaching significance versus controls at 72 hrs postburn ($p<0.05$). Associated with this increase of MEL levels at 72hrs postburn was an enhanced splenocyte production of inflammatory

cytokines, including IL-1, IL-6, IFNγ, while splenic IL-2, IL-4 and IL-10 levels were not different between control and injured groups. **Conclusions:** These results suggest that the higher daytime MEL levels early postburn in young guinea pigs and mice may reflect an adaptive response by the injured host to meet the oxidative challenge of injury by increasing serum levels of MEL. Further evaluation of the inability of the older injured guinea pigs to mount this early response is warranted to assess whether lower MEL levels in this population is related to increased risk for adverse outcome.

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NOREPINEPHRINE RELEASE IS INCREASED IN BONE MARROW FOLLOWING THERMAL INJURY.
Y.Tang*, R.Shankar*, S. Santangelo*, R.Gamelli and S.Jones. Loyola Univ. Med. Center, Maywood, IL 60153
Evidence for increased sympathetic activation following thermal injury is well established. Recent pharmacologic data suggest that adrenergic mechanisms are involved in myelopoiesis. Previously we have established that myelopoiesis is profoundly altered following thermal injury with sepsis. Together, these facts indicate that adrenergic stimulation may regulate the pathophysiologic changes in myelopoiesis following thermal injury. To test this premise, the dynamic modulation of norepinephrine (NE) release following thermal injury and sepsis must be ascertained. Therefore the present study was designed to determine the bone marrow (BM) sympathetic nerve activity in response to thermal injury with sepsis. Mice (B6D2F1/J) were subjected to a 15% scald surface burn alone or burn injury to which 1000 cfu of *Pseudomonas aeruginosa* were applied topically at the wound site. Sympathetic activation was estimated on days 1 and 3 after the initial injury by NE turnover measurements where tissue specific activity ([^{3}HNE]/[NE total]) was followed by chasing a pulse of [^{3} HNE]. BM turnover rate was 0.39 ·24 hr after burn plus sepsis which was significantly greater than sham level of 0.28 ng/g/hr. At day 3 with burn plus sepsis turnover of 0.46 was significantly greater than sham of 0.20 ng/g/hr. At both times BM turnover of NE was not different in burn without sepsis compared to sham. These results demonstrate that sympathetic activation is markedly increased in BM following burn plus sepsis thus setting the stage to explore the cause-effect relationship between adrenergic stimulation and myelopoietic alterations. Supported by MH53562 (SJ) and GM56424 (RS).

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EFFICACY OF HYPERTONIC SALINE DEXTRAN FLUID RESUSCITATION FOR PATIENTS WITH HYPOTENSION FROM PENETRATING TORSO INJURIES
C.E. Wade, J.J. Grady*, M.J. Wall*, G.C. Kramer, Medisan Pharmaceuticals Inc., Uppsala, Sweden, Dept. of Biostatistics UTMB, Galveston TX. Dept. Surgery, Ben Taub Hospital, Houston, TX. Dept. Anesthesiology, UTMB, Galveston TX.

The purpose of this study was to assess if the administration of Hypertonic Saline Dextran (HSD) was detrimental when administered to patients who were hypotensive due to penetrating torso injuries. We prospectively designed a series of questions to be addressed by a meta-analysis of individual patient data using computerized data files and case report forms from a multi-center study of HSD. The investigators were 'blind' as to the treatment the patient received. Patients (n=230) with penetrating torso injuries were studied as to survival until discharge. The patients were administered 250 ml of HSD or normal saline (standard of care, SOC) as the initial fluid therapy. Of the 120 patients treated with HSD 82.5% survived compared to 75.5% for 110 SOC patients (p=0.189). Sixty-eight percent (n=157) of these patients required surgery. HSD treatment (n=84) in this population improved (p=0.010) survival, 84.5% compared to 67.1% with SOC (n=73). HSD resulted in an increase in blood pressure and a reduction in hematocrit, with no differences noted in fluid requirements or indices of clotting. For patients with penetrating torso injuries that result in hypotension initial fluid resuscitation with HSD is beneficial in improving survival, especially if surgery is subsequently required.

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SIGNIFICANCE OF PROCALCITONIN (PCT) PLASMA LEVELS DURING THE CLINICAL COURSE OF SEVERELY INJURED PATIENTS.
G.A. Wanner, M. Keel, R. Stocker, O. Trentz, W. Ertel. Division of Trauma Surgery, University Hospital of Zurich, Zurich, Switzerland

Severe trauma is associated with a high incidence of septic complications and multiple organ dysfunction syndrome (MODS) which markedly influence outcome of injured patients. Standard clinical and laboratory markers of systemic inflammation (fever, leukocytosis, C-reactive protein) are unspecific and do not allow the differentiation between infectious and non-infectious systemic inflammatory response syndrome (SIRS). Though high concentrations of PCT have been found in patients with severe generalized infection, the diagnostic and prognostic value of routine PCT measurements for the management of multiple injured patients remains to be determined. 405 patients with mechanical trauma (ISS > 9 pts) were enrolled in this study. PCT plasma levels were determined using a specific immunoluminometric assay at the day of admission and on days 1, 3, 5, 7, 10, 14, 21 thereafter and compared with the severity of injury (ISS) and the incidence of MODS, SIRS, and sepsis. Data are mean ± SEM; one-way ANOVA and Student-Newman-Keuls test. Mechanical trauma led to increased PCT plasma levels dependent on the severity of injury with peak values on day 1 and 3 ($p<0.05$) and a continuous decrease within 21 days. Patients developing SIRS revealed a significant ($p<0.05$) increase of peak PCT plasma levels compared with patients without SIRS. Highest PCT plasma concentrations early after injury were observed in patients with sepsis (6.9 ± 2.5 ng/mL; day 1) or severe MODS (5.7 ± 2.2 ng/mL; day 1) with a sustained increase ($p<0.05$) for 14 days compared to patients with uneventful posttraumatic course (1.1 ± 0.2 ng/mL). Moreover, these increased PCT plasma levels during the first three days after trauma predicted ($p<0.0001$) severe SIRS, sepsis, and MODS. These data indicate that PCT represents a sensitive and predictive indicator of sepsis and severe MODS in injured patients.

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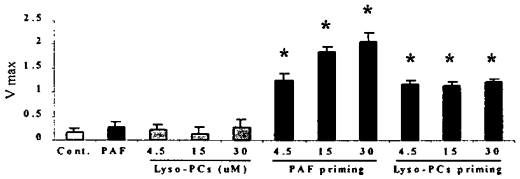
THE ROLE OF TRANSFUSIONS AND THE TWO HIT MODEL OF MOF. J. Aiboshi*, E. Moore, G. Zallen*, D. Ciesla* and C. Silliman*. Denver Health Medical Center, Denver, CO 80204.

Stored blood contains lysophosphatidylcholines (lyso-PCs) which are biologically active and may serve as the second hit. Our previous studies show that circulating neutrophils (PMNs) are primed by multiple agents, especially platelet-activating factor (PAF) at an early time after trauma and transfusions are correlated with multiple organ failure (MOF). We **hypothesize** that cytotoxicities of primed PMNs following trauma will be augmented by transfusions; the purpose of this study is to examine the direct effect of transfusions on PMN function. **Methods:** PMNs were isolated by density gradient centrifugation and primed for 5 min with buffer, PAF (2 μM) or lyso-PCs (4.5, 15, and 30 μM) followed by stimulation with lyso-PCs (4.5, 15, and 30 μM) or PAF (2 μM). Superoxide (O_2^-) was measured by the SOD-inhibitable reduction of cytochrome c. Data are expressed as the V_{max} of O_2^- generation; mean ± SEM, *p<0.05 from control by ANOVA/Fisher's PLSD. **Results:** Neither PAF nor lyso-PCs alone produced O_2^-. PAF priming followed by lyso-PCs significantly increased O_2^- production in a dose-dependent manner. In addition, lyso-PCs primed for PAF activated O_2^- production.



Conclusion: Lyso-PCs primed resting PMNs and activated PAF primed PMNs for O_2^- production. Thus lyso-PCs contained in stored blood may augment PMN cytotoxicity.

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DIFFERENTIAL REGULATION OF Ca^{2+} INFLUX BY FMLP AND PAF IN HUMAN NEUTROPHILS: POSSIBLE INVOLVEMENT OF STORE-OPERATED Ca^{2+} CHANNEL L.-W. Chen*, J.-S. Chen* and S.-N. Wu*, (Spon:M.-S. Liu). Veterans General Hospital-Kaohsiung, Kaohsiung, Taiwan.

Ca^{2+} influx into human polymorhponuclear cells (PMNs) in response to fMLP and PAF stimulation was studied. Whole blood was taken by venous puncture from healthy human volunteers. PMNs were isolated, diluted and incubated with 2 μl/ml fura-2 AM for 20 min at 37°C. The cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$, in human neutrophils was determined by microfluorometry. We found the initial rising of fluorescence curves of PMN intracellular Ca^{2+} levels after the stimulation by PAF or fMLP was preserved after the addition of EGTA, but the slope and the peak hight of fluorescence curves were declined to about 2/3 of the curves without EGTA. Treatment of PMN with PMA completely abolished intracellular Ca^{2+} stimulated by PAF, but not the intracellular Ca^{2+} level stimulated by fMLP. Treatment of PMN with PAF did not abolish the intracellular Ca^{2+} level stimulated by fMLP. In addition, treatment of PMN with fMLP did not abolished intracellular Ca^{2+} stimulated by PAF. Loperamide elicited an increase in intracellular calcium after the activation of SOC channels stimulated by fMLP or PAF. After the addition of dbGMP, the initial increase of of PAF or fMLP induced PMN intracellular Ca^{2+} fluorescences were well preserved, but the slope and the peak hight of fluorescence curves were declined in comparison to the curves without dbGMP. In conclusion, we found PAF and fMLP regulate the Ca^{2+} influx of PMN with different ways. The initial mobilization of intracellular Ca^{2+} stores in PAF stimulated route is mediated by PKC phosphorlyation, but not in fMLP stimulated route. SOC is present and important in the fMLP or PAF induced PMNs Ca^{2+} influx. There was no apparent cross-regulation between PAF and fMLP.

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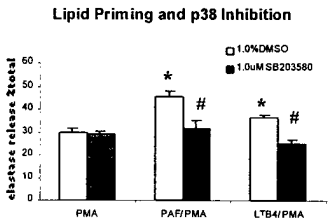
LIPID PRIMING FOR PMN ELASTASE RELEASE REQUIRES p38 MAPK DJ Ciesla*, EE Moore, G Zallen*, WL Biffl, CC Silliman* Denver Health Medical Center, Denver, CO 80204

PMN priming is pivotal in the development of postinjury MOF. PMN derived elastase is capable of cytotoxicity independent of superoxide production. The lipid mediators PAF and LTB4 play a central role in postinjury PMN priming for elastase release. Both PAF and LTB4 activate p38 MAPK. The purpose of this study was to investigate the role of p38 MAPK in PAF/LTB4 priming of PMN for elastase release. **METHODS:** Human PMN were isolated by dextran sedimentation and density gradient centrifugation then incubated with the p38 specific inhibitor SB203580 (1.0μM) or vehicle control (1.0% DMSO) for 20min at 37°C. PMN were then primed for 5min with PAF or LTB4 and activated with PMA. PMA promotes PMN elastase release independent of p38 activation. Elastase content of the resultant supernate was measured by cleavage of AAPV-pNitroanilide. Elastase release is expressed as %total of cell lysate (mean+/-SEM). **RESULTS:** PAF/LTB4 augmented PMA mediated elastase release from 29% to 45% and 36% respectively (*p<.05). Inhibition of p38 had no effect on PMA mediated elastase release in non-primed PMN. However, p38 inhibition decreased PMA mediated elastase release in PAF/LTB4 primed cells from 45% and 36% to 32% and 25% respectively. (#p<.05) **CONCLUSIONS:** Inhibition of p38 MAPK blocks PMN lipid priming for elastase release and offers a potential target for the reduction of postinjury PMN cytotoxicity.



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CXC CHEMOKINES REGULATE PMN APOPTOSIS THROUGH CASPASE-3 SUPPRESSION WHICH PRESERVES THEIR FUNCTION. A. Dunican*, S.J. Leuenroth*, A. Ayala and H.H.Simms. Brown University/Rhode Island Hospital, Dept. of Surgery, Providence, RI 02903

CXC chemokines, such as IL-8 and Gro-α, delay PMN apoptosis(A_o) which is thought to be important in the resolution of inflammation. Our hypothesis is that IL-8 and Gro-α suppress neutrophil A_o via an intracellular mechanism which involves caspase regulation. Additionally, we sought to determine if prolonged survival correlated with functional significance. **Methods**: PMN cultured with IL-8 or Gro-α(0-100ng/ml) were assessed for A_o. To induce A_o, TNF-α(100ng/ml) or FasL(50ng/ml) were added to PMN in the presence or absence of IL-8 or Gro-α. Caspase-3 activity was measured by caspase-specific substrate cleavage. In addition, PMN function was determined by O_2^- generation after PMA(10ng/ml) stimulation. **Results**: PMN A_o was suppressed maximally at 100ng/ml from 48±4% to 5±3% and 6.5±4% at 12 hours

for control PMN, IL-8 and Gro-α, respectively (n=9, p<0.0001). At 4 hours, PMN cultured with TNF-α and IL-8 or Gro-α have a decrease in A_o from 11±1.5 for TNF-α alone to 2.7±0.2 and 2.8±0.6 for TNF-α+IL-8 and TNF-α+Gro-α (n=3, P<0.0001). At 12 hours, PMN cultured with FasL showed a 30% increase in A_o versus PMN cultured alone. PMN cultured with FasL+IL-8 or Gro-α had no increase in A_o over PMN cultured with IL-8 or Gro-α alone. Specific caspase activity assays demonstrated that IL-8 and Gro-α suppressed activation of caspase-3 at 24 hours: caspase-3 activity was 67±14, 8.6±0.8 and 20±7 (pmol/min/ cell #x10^{-7}) for control cells, PMN+IL-8 and PMN+Gro-α, respectively(n=3, P<0.0005). IL-8 and Gro-α preserved function in viable neutrophils. After stimulation with PMA, PMN cultured with Gro-α or IL-8 produced 0.25nM and 0.23 nM O_2^- /1x 10^6 PMN, respectively while control cells produced only 0.05 nM O_2^- /1 x10^6 PMN(n=3, p<0.002). **Conclusion**: IL-8 and Gro-α delay PMN A_o, which is unchanged by the addition of apoptosis inducers TNF-α or FasL. Chemokine induced delay in A_o appears to be mediated by inhibiting caspase-3 activation which leads to retention of PMN functional activity. These chemokines which are found in inflammatory tissues may be important in the pathogenesis of disease states, such as ARDS or SIRS.

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PREVENTION OF GUT NEUTROPHIL SEQUESTRATION AND BACTERIAL TRANSLOCATION BY ANTI-CINC ANTIBODY IN BURN INJURED RATS. N. Fazal, M. A. Choudhry, S. Khan*, T. Ravindranath & M. M. Sayeed, Departments of Physiology and Surgery, and Burn and Shock Trauma Institute, Loyola University Chicago, Maywood, IL.

Anti-cytokine-induced neutrophil chemoattractant (CINC) antibody has been shown to be a potent blocker of neutrophil chemotaxis and infiltration. It may block neutrophil related host tissue injury in the early stages of burn inflammation. In this study, we investigated potential relationship between gut neutrophil influx and the occurrence of mucosal bacterial translocation (BT) in burn-injured rats (3rd degree burn, 30% TBSA). The rats were divided into three experimental groups, Group 1, injected intravenously with rabbit anti-CINC antibody (1 mg/ml phosphate-buffered saline); Group 2, injected with rabbit sera without antibody, and group 3 with saline only. Myeloperoxidase activity (MPO) determined the sequestration of neutrophils in rat intestine, and specific PCR and Southern blot analysis was performed to detect Escherichia coli invasion into intestinal and extra-intestinal tissue sites. The results showed that pretreatment with anti-CINC-Abs blocked BT more pronouncedly in the small intestine tissue (INT), and Mesenteric lymph nodes (MLN) and less so in Peyer's patches (PP) of burned rats.

Percentage reduction in Bacterial Translocation:

	INT		MLN		PP	
Animal No:	1	2	1	2	1	2
1. Burn day 1	86	81	55	50	20	25
2. Anti-CINC	44	52	15	20	16	22

Values represent densitometric unit changes as percent of E.coli (10^9) positive controls.

The data suggest that neutrophil infiltration plays an a role in the onset of bacterial translocation. Because the blockage of the action of CINC appears to prevent BT, BT may be related to neutrophil infiltration. (Supported by NIH grants GM 53235 and GM 568501).

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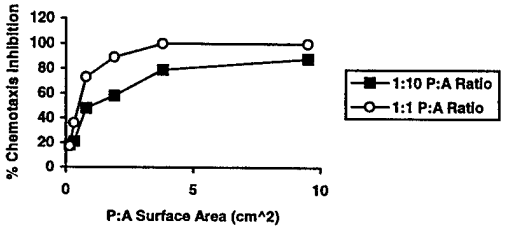
INHIBITION OF NEUTROPHIL CHEMOTAXIS BY PERFLUBRON. R.Fernandez*,J.Younger*,R.Hirschl*, P.Ward. Univ. Michigan, Ann Arbor, MI 48109-0303.

Objective: Previous experiments have shown that partial liquid ventilation with perflubron decreases lung neutrophil (PMN) accumulation in several models of acute lung injury. We explored in vitro this phenomenon, hypothesizing that perflubron would inhibit PMN chemotaxis. We specifically considered volume and surface relationships. Isolated human PMN were incubated for 5 min with varying perflubron:aqueous (P:A) volumes, aspirated from the perflubron surface, and allowed to migrate to a 4nM fMLP stimulus in a micro Boyden chamber. In separate experiments, PMN suspensions were layered on perflubron-containing polystyrene wells of varying P:A diameter, then placed in a Boyden chamber as above. Chemotaxis was quantified by counting migrated cells in 5 high power fields. PMN chemotaxis was inhibited by perflubron in a volume-dependent manner, with 50% inhibition seen at the 1:100 P:A mixture and complete inhibition achieved with a 1:1 mixture (p<0.01). Inhibition was directly related to the P:A boundary surface area (see figure, p<0.01). Perflubron exposure did not reduce cell viability (by Trypan Blue exclusion). Increasing doses of fMLP did not overcome the inhibitory effect. Conclusion: Perflubron inhibits the in vitro migration of PMN in both a volume- and surface area-dependent manner. This in part explains the decreased lung PMN content seen during liquid ventilation in vivo and suggests a contact-dependent mechanism.



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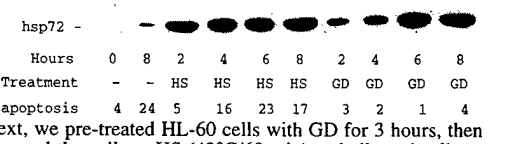
HEAT CAUSES APOPTOSIS IN PHAGOCYTES INDEPENDENTLY OF HSP72 PRODUCTION. J. Horn, T. Callahan*, and W. Welch*, San Francisco General Hospital, UCSF, San Francisco, CA 94143-0807.

Human polymorphonuclear leukocytes (PMN) exposed *in vitro* to heat shock (HS) produce heat shock proteins (HSP) and are rendered prematurely apoptotic and functionally inactive, suggesting that mild heat is anti-inflammatory and not cytoprotective as expected. To resolve this conundrum, we hypothesized that HS-induced apoptosis occurs independently of HSP production in phagocytic cells. To test this, we substituted HL-60 cells for PMN and examined induction of HSP72 production and apoptosis after exposure to two alternative stresses. This Western blot was obtained from aliquots of cell lysates probed for HSP72 following exposure to either 100 ng/ml geldanamycin (GD) or heat shock (43°C/60 min) and recovered for 8 hours. Below each lane are rates of apoptosis (annexin$^+$/propidium iodide$^-$) measured by flow cytometry. Findings were duplicated (n=6).



Next, we pre-treated HL-60 cells with GD for 3 hours, then exposed the cells to HS (43°C/60 min) and allowed cells to recover for 24 hours. Displayed are mean ± SEM.

Pre-treatment (3 hr)	2° Stimulus	% Apoptosis (24 hr)
None	HS	21.9 ± 2.1
100 ng/ml GD	none	4.5 ± 0.6
100 ng/ml GD	HS	9.8 ± 1.1*

*p < 0.05 vs. no GD (HS alone), Student's t-test.

Similar to our previous findings with PMN, HL-60 cells were rendered prematurely apoptotic with HS exposure, whereas GD failed to cause significant apoptosis. Yet, both HS & GD induced production of potentially cytoprotective HSP72. In addition, pre-induction of HSP production by GD, conferred > 50% cytoprotection against subsequent heat-induced apoptosis. We conclude that the anti-inflammatory phagocyte apoptosis caused by heat exposure, occurs independently of HSP production and can only be prevented by prior HSP induction.

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PANCREATIC NEUTROPHIL ACTIVATORS IN SAO SHOCK. E. B. Kistler*, H. Mitsuoka*†, G. W. Schmid-Schönbein*†, and T. E. Hugli*, (Spon: A. Lefer). Department of Immunology, The Scripps Research Institute, †Microcirculation Laboratory, Department of Bioengineering and Institute for Biomedical Engineering, University of California, San Diego, La Jolla, CA.

Plasma factors from splanchnic arterial occlusion (SAO) shock result in upregulated levels of cellular activation, as measured by pseudopod formation tests in donor neutrophils exposed to shock plasma. We hypothesize that these plasma activators may be produced in endogenous tissue. We have previously found that homogenates made of rat peritoneal organs do not significantly activate isolated naive neutrophils except for pancreatic homogenate. After application of pancreatic proteases, however, these organ homogenates produce activators. We wanted to determine whether fluid from the pancreas can transport cellular activating factors such as are seen in SAO shock. Rats were weighed, anesthetized and catheterized. After laparotomy, the superior mesenteric and celiac arteries were clamped for 100 minutes, followed by unclamping and reperfusion. The pancreas was immediately removed and snap-frozen at -70°C. The pancreatic tissue was homogenized in 1:3 (w/v) saline solution and incubated for 2.5 hours at 38°C. In control experiments, rat bile and pancreatic ducts were cannulated and fluid collected. Bile fluid and effluent from the pancreatic duct were incubated either in the presence or absence of trypsin, which has previously been shown to produce neutrophil activating factors in organ homogenates. All samples were measured for their ability to induce neutrophil pseudopod formation. The pancreatic homogenates we tested induced significant pseudopod formation compared to controls ($p<0.001$), as did pancreatic duct fluid, both in the presence ($p<0.05$) and absence of trypsin ($p<0.05$). Bile fluid, bile fluid incubated with trypsin, and control saline incubated with trypsin did not significantly activate neutrophils. These results indicate that activating factors may reach the systemic circulation via the gut. The pancreas may serve as an endogenous source for the production of cell activators. Support by U.S.P.H.S. Grant HL-43024, AI-41670, and HL-07195.

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ANTISENSE OLIGONUCLEOTIDES AGAINST MCL-1 RESULT IN INCREASED APOPTOSIS OF HUMAN NEUTROPHILS. S.J. Leuenroth*, P.S. Grutkoski*, A. Ayala, and H.H. Simms. Brown University/ Rhode Island Hospital, Dept. of Surgery, Providence, RI 02903.

The regulation of neutrophil apoptosis can greatly influence an inflammatory response, however the mechanisms responsible for PMN viability are not well understood. **Purpose:** Previous studies in our laboratory have found neutrophils to express Mcl-1, a Bcl-2 family member, by both Western blot analysis and RNAse protection assays. We further sought to characterize the function of Mcl-1 by localization and antisense studies. **Methods:** For immunofluorescent staining, PMN were harvested between 4 and 20 hours of incubation, fixed, permeabilized, labeled with an anti-Mcl-1 antibody, and viewed by confocal microscopy. For antisense experiments, the following modified oligo constructs were designed: Antisense: GGGGCTTCCATCTCCTCAA Sense: CCCCGAAGGTAGAGGAGTT. PMN were incubated with 5μM of either the sense or antisense oligo for 3 hours in serum free RPMI, washed, and then incubated for a total time of 8 or 12 hours in supplemented RPMI. **Results:** Freshly isolated PMN exhibited strong Mcl-1 staining within the nucleus as well as the cytoplasm, whereas 4 and 8 hours of incubation resulted in slight nuclear but predominantly cytoplasmic and peri-nuclear staining. In contrast, by 20 hours Mcl-1 was completely absent in apoptotic cells, whereas localization was only cytoplasmic in viable cells. Antisense experiments showed that after 8 and 12 hours of adherence in the presence of Mcl-1 antisense oligo, there was a significant increase in PMN apoptosis over both the sense construct and media alone (8 Hour Data: % Apoptosis for antisense: 44.6 ± 3.2 vs. 15.6 ± 2.8 (sense) or 14.7 ± 2.0 (media) 12 Hour Data: antisense: 64.7 ± 1.5 vs. 45.6 ± 0.4 (sense) or 43.2 ± 1.8 (media), $p< 0.0001$, N=3).

Conclusions: The anti-apoptotic protein, Mcl-1, is present in mature human neutrophils. Here, we have found localization of this protein to both the nucleus and membrane bound organelles, as well as describing a functional role in preserving neutrophil viability. The regulation of Mcl-1 may potentially serve as a target in modulating neutrophil half-life during the inflammatory response.

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INTESTINAL LAVAGE INCREASES SURVIVAL AFTER SAO SHOCK. H. Mitsuoka*, E. Kistler*, and G. W. Schmid-Schönbein*, (Spon: A. Lefer). Microcirculation Laboratory, Department of Bioengineering and Institute for Biomedical Engineering, University of California, San Diego, La Jolla, CA.

Splanchnic arterial occlusion (SAO) shock is a shock model with uniformly high mortality, and is accompanied by upregulated levels of cellular activation as measured by pseudopod formation and NBT tests in donor neutrophils exposed to shock plasma. This activation has been found to be inhibited by protease inhibitors, which have also mitigated the systemic effects of SAO shock. In vitro experiments on organ homogenates has identified proteolytically derived factors from the pancreas as a source of cellular activating factors. We were interested in determining the importance of pancreatic proteases and their products in the injury seen to the gut and systemically during SAO shock. Rats were divided into SAO shock, SAO shock with intestinal lavage, SAO shock with pancreatic ligation, and sham shock groups. SAO shock was induced by clamping the superior mesenteric and celiac arteries for 100 minutes, followed by unclamping and reperfusion. SAO shocked animals had significantly lower survival rates ($p<0.01$), MAP after reperfusion ($p<0.01$), and elevated neutrophil activation ($p<0.01$) compared to sham shock animals which was not reversed by pancreatic ligation. Continuous intestinal lavage of SAO shocked animals, however, resulted in significant increases in survival time ($p<0.01$), MAP ($p<0.01$), and lower cellular activation ($p<0.01$), compared to the SAO shock group. These results indicate that activating factors for circulating leukocytes and endothelial cells may reach the systemic circulation via the intestine in SAO shock. Support by U.S.P.H.S. Grant HL-43024.

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OPTICALLY ACCESIBLE MICROCHANNELS REVEALED THAT LPS, IL-1β AND G-CSF DECREASE NEUTROPHIL DEFORMABILITY IN ANESTHETIZED RATS. M. Nishino*, H. Tanaka* and H. Sugimoto*, (Spon:H. Hirasawa) . Osaka University Medical School, Osaka 565-0871, Japan.

The effects of intravenous administration of LPS, IL-1β and G-CSF on the blood rheology were examined in anesthetized rats. LPS (100 μg/kg, n=6), IL-1β (5 μg/kg,n=6) and G-CSF(5 μg/kg,n=3) were administered intravenously and blood samples were collected at 1 and 2 hours after the injection. By using a novel microchannel array etched on the single-crystal silicon tip which is simulated the microvasculature, the time taken for 100 μl of whole blood to pass through the microchannnel was determined. The alterations of neutrophil deformability and platelet aggregability were observed under a microscope attached with a video camera. The number of white blood cell at 1 hour after LPS and IL-1β decreased significantly compared with baseline (LPS: 5270 ± 1800 to 1780 ± 530 /mm^3 ,$p<0.01$ and IL-1β:

6700 ± 850 to 4970 ± 450 /mm^3, p<0.05, mean ± SD). G-CSF did not change the number of white blood cell at even 2 hours after injection. The whole blood transit time after LPS and IL-1β increased significantly compared with baseline (LPS: 28.0 ± 3.2 to 35.5 ± 3.7 sec/100μl, p<0.01 and IL-1β: 33.1 ± 1.8 to 38.0 ± 5.1 sec/100μl, p<0.01). G-CSF did not significantly prolong the whole blood transit time. Increased adhesiveness and stiffening of neutrophils as well as increased aggregability of platelet were observed at 1 and 2 hours after LPS, IL-1β and at 2 hours after G-CSF. Many microchannels were obstructed by neutrophils decreased their deformability, which contributed to the prolonged whole blood transit time. These findings may suggest that activated neutrophils reduce the effective blood flow in the microcirculation, which is one of the mechanisms that those neutrophils develop tissue injury.

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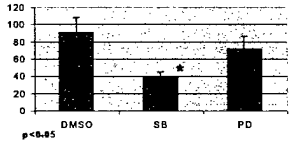
MAPK REGULATION OF PMN TNF-α PRODUCTION. B. Nolan*, R. Furse*, A. Duffy*, L. Paquin*, M. De*, C.Miller, P. Bankey. Univ. of Massachusetts. Med. School, Worcester, MA.

Cytokines including TNF-α mediate local and systemic inflammatory responses. MAPKs are intracellular signaling pathways which process extracellular stimuli. We hypothesize that neutrophil TNF-α production in response to bacterial endotoxin (LPS) is mediated by MAPK signal transduction.

PMNs were isolated from the blood of healthy volunteers (n=8). Cells were treated with either ERK inhibitor (10μM PD98059), p38 inhibitor (1μM SB20358) or vehicle alone (DMSO 0.1%). Cells were then stimulated with 1-μg/ml LPS. Activation of p38, ERK and SAPK/JNK MAPKs were determined by western blot analysis. TNF- α mRNA levels were measured by ribonuclease protection assay (RPA). TNF-α secretion was determined by ELISA. Cell viability was determined by cell death ELISA. Data were normalized and compared by ANOVA.

Fig 1. Secreted TNF-α (pg/ml)



LPS activated p38 and p42/p44ERK MAPKs with maximal phosphorylation occurring at 30 minutes. Activation of SAPK/JNK did not occur. TNF-α message levels also peaked 30 minutes following stimulation. Inhibition of p38 with SB significantly blocked TNF-α secretion at 18-hours. ERK inhibition had no significant effect on TNF-α secretion (**Figure 1**). TNF-α message levels at 30 minutes were unaffected by inhibitor pretreatment. In conclusion, p-38 MAPK is a post-transcriptional regulator of human neutrophil TNF-α secretion following LPS stimulation. Delineation of MAPK function in PMNs may contribute to the understanding and treatment of inflammatory syndromes.

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NEUTROPHIL OXYGENATION ACTIVITY IN NORMAL AND DIABETIC BABOONS (*PAPIO ANUBUS*). G. Peer*, M.E. Cary, R.C. Allen*, A.C.K. Chang, D. Carey, III, F. B. Taylor, Jr. Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

The blood phagocyte function of a diabetic baboon was tested and compared to normal baboons using a whole blood leukocyte luminescence assay. Oxidase-dependent luminescence activity (Ox) was stimulated with low dose PMA (20 pmol/tube; 33nM final) and oxidase-dependent myeloperoxidase (Ox-MPO) activity was stimulated with high dose PMA (5 nmol/tube; 8.3 μM final). Luminol was used as the chemiluminigenic substrate. Following dilution, the activity of 0.5 μL of blood was measured at 10 and 20 minutes. Blood phagocyte activities were measured in 4 normal baboons and 1 diabetic baboon (run in duplicate). The luminescence activity was normalized per phagocyte in the blood specimens tested. Basal (B), Ox, and Ox-MPO activities were measured at 20 minutes and the results are expressed as the fold increase relative to basal activity:

	B	Ox	Ox-MPO
Normal	1 ± 0.2SEM	3.3 ± 0.6SEM	9.0 ± 1.5SEM
Diabetic	1 ± 0.2SEM	13.3 ± 2.2SEM	33.0 ± 5.3SEM

Luminol-dioxygenation luminescence activity reached a maximum by 20 minutes and then slowly declined. The preliminary data suggest substantially elevated Ox and Ox-MPO activities in blood phagocytes from the diabetic baboon. Functional alteration may reflect an increased *in vivo* exposure to inflammatory agents and/or increased myelopoietic activity. These differences may also be related to increased membrane glycosylation or altered sorbitol pathway metabolism.

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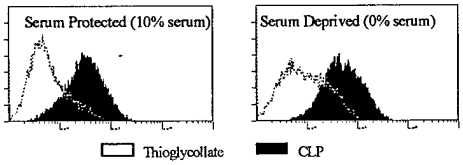
ENHANCED APOPTOSIS IN ACTIVATED PERITONEAL NEUTROPHILS DURING FECAL PERITONITIS. M.S.Shrotri*, J. Kuhn*, G. Franklin*, J.Peyton*, and W.G.Cheadle. VAMC and the Department of Surgery, University of Louisville, Louisville, KY 40292.

Introduction: Neutrophil (PMN) apoptosis is a key event in resolution of tissue inflammation. In this study we determined the role of bacteria by comparing peritoneal (activated) neutrophil apoptosis in fecal peritonitis and thioglycollate (non-bacterial) peritonitis. **Materials and Methods:** Swiss Webster mice (n=8) underwent cecal ligation & puncture (CLP) or intraperitoneal (i.p.) thioglycollate (n=8) administration. 18 h later peritoneal cells (PECs) were harvested. Cytospins were performed for differential PEC counts. PECs were incubated over 24 h with serum (protected) or without serum (deprived). Cells were then fixed and permeabilized. Apoptotic cell population was assessed with flow cytometry after TUNEL procedure. DNA laddering was also performed to detect apoptosis in PECs. **Results:** *1. Peritoneal influx*—Peritoneal PMN influx was similar after CLP and i.p. thioglycollate at all time-points, with >90% cells being PMNs. *2. Apoptosis in PECs*—Apoptosis was delayed in the serum protected thioglycollate group (14%), and did not increase significantly in the serum deprived group(17%). In contrast, more cells were induced to undergo apoptosis in the serum protected CLP group (35%), and the numbers increased further in the serum deprived group (48%), indicating the absence of protection offered by activation. *3. DNA laddering*—Gels confirmed the differential apoptosis observed in PECs from the two models.



Conclusion: Although the PMNs are activated in both groups, the apoptosis response is different in bacterial peritonitis as compared to non-bacterial peritonitis. Peritoneal PMNs in the thioglycollate group showed delayed apoptosis in both serum protected and deprived groups. In the CLP animals, ingestion of bacteria induced more activated cells to undergo apoptosis in both serum protected and deprived groups. Local presence of bacteria, thus, affects the apoptosis process in the activated PMNs.

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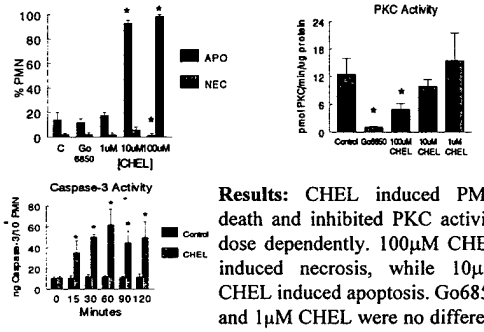
CHELERYTHRINE CHLORIDE INDUCES RAPID PMN APOPTOSIS THROUGH ACTIVATION OF CASPASE-3 J.F. Sweeney*, P.K. Nguyen*, K.B. Atkins* and D.B. Hinshaw University of Michigan and Ann Arbor VAMC, Ann Arbor, MI 48105

Protein kinase C (PKC) plays a role in suppression of apoptosis in many cell lines. **Purpose:** To determine the effect of PKC inhibitors chelerythrine chloride (CHEL) and bis-indolylmalyeimide (Go6850) on PMN apoptosis. **Methods:** PMN were cultured with 10 fold serial dilutions of CHEL (100μM-1μM) or 10μM Go6850. Apoptotic and necrotic PMN were determined at 4hr using fluorescence microscopy with acridine orange/ethidium bromide staining. PKC activity was measured using a PKC assay system. Caspase-3 activity induced by 10μM CHEL was measured fluorometrically. Data are the mean ± SEM of 4 experiments. * = different from control, p<0.05 ANOVA.



Results: CHEL induced PMN death and inhibited PKC activity dose dependently. 100μM CHEL induced necrosis, while 10μM CHEL induced apoptosis. Go6850 and 1μM CHEL were no different than control. Go6850 and 100μM CHEL inhibited basal PKC activity but not 10μM or 1μM CHEL. 10μM CHEL significantly increased caspase-3 activity after only 15 minutes of incubation. **Conclusions:** CHEL rapidly activates caspase-3 and induces apoptosis in human PMN. This appears to occur via a PKC-independent mechanism.

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EFFECTS OF INDUCED HYPOTHERMIA ON SYMPATHETIC NERVE ACTIVITY, BARORECEPTOR REFLEX IN URETHANE-ANESTHETIZED RABBITS. M. Aibiki, H. Xu, K. Seki, S. Ogura, S. Yokono and K. Ogli. Dept. of Anesthesiol. and Emerg. Med., Kagawa Medical Univ., 1750-1, Ikenobe, Miki, Kita, Kagawa, 761-0793, Japan.

To evaluate the role of the autonomic nervous system in hemodynamic changes during induced hypothermia, we examined the effects of surface cooling on heart rate (HR), mean blood pressure (MBP) and renal sympathetic nerve activity (RNA) in urethane-anesthetized rabbits using direct recordings of RNA. Changes of baroreflex sensitivity and plasma catecholamines (epinephrine; E, norepinephrine; NE) were also measured simultaneously. The animals were divided into four groups: animals with an intact neuraxis (intact group; n=8), cervical vagotomized animals (vagotomy group; n=6), sino-aortic denervated animals (SAD group; n=6), and animals with SAD plus vagotomy (SADV group; n=6). Surface cooling caused progressive and profound decreases in HR in all groups. An initial increase in RNA, followed by a depressive response, occurred even from 36°C in esophageal temperature (ET). This augmented RNA response was not associated with significant changes of MBP until 32°C. However, hypotension developed when ET reached 26°C. These responses were similarly found in the other three groups. In the intact group, at 34°C and 30°C, barosensitivities evaluated by HR were significantly suppressed, but those by RNA were preserved completely (n=5). Plasma E and NE levels in the intact group made a peak at 30°C, but returned to near the control level at 22°C. These results suggest the following: 1) Hemodynamics and RNA during induced hypothermia are regulated by mechanisms other than the baroreflex reflex system, possibly such as cold receptors. 2) Suppression of the baroreflex occurred on HR but not on RNA during hypothermia, which may indicate the direct effects of hypothermia on the heart.

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NFκB ACTIVATION IN MOUSE HEART AFTER LPS AND/OR TNFα STIMULATION. S.B. Haudek*, D.D. Bryant*, J.W. Horton and B.P. Giroir. Univ. of Texas SWMC, Dallas, TX 75235.

Nuclear factor kappa B (NFκB) regulates transcription of many genes, such as TNFα, which contribute to myocardial dysfunction. The purpose of this study was to determine the time course and dose response of NFκB activation as well as mechanisms of its inhibition in mouse heart. After LPS challenge (1 mg/kg) NFκB was activated in the heart as early as 30 min, continuing for 8 h. Peak upregulation was between 1 to 2 h. Administration of 6 mg/kg LPS activated cardiac NFκB at 15 min, continuing up to 36 h. TNFα (25 μg/kg) induced NFκB translocation in mouse heart 30 min to 4 h after challenge. The time course of IκBα degradation as determined by western blot confirmed these kinetics. Blockade of TNFα bioactivity with a dimeric receptor protein (rhu TNFR:Fc, Immunex) failed to inhibit cardiac NFκB activation after LPS challenge, but blocked activation after TNFα administration. To further examine if rhu TNFR:Fc could regulate TNFα locally produced in the heart, we treated transgenic mice having cardiac TNFα constitutively overexpressed. In control transgenics myocardial NFκB was activated at all ages tested (21d, 40d, 75d). Within 2 h after administration of rhuTNFR:Fc (5 mg/kg) NFκB translocation was reduced, blocked totally after 4 h, and increased back to full activity after 18 h. These data indicate that the heart rapidly responds to LPS and TNFα. Furthermore, they suggest that the heart directly responds to LPS since myocardial NFκB activation is not due to LPS - induced TNFα production.

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ROLE OF THE ENDOTHELINSYSTEM IN NORMAL AND HYPERTROPHIC RAT HEARTS. R. Meier*, H. Tscherne* and H. Schunkert* (Spon: HJ. Oestern) Hannover Medical School, D-30625 Hannover and University of Regensburg, D-93042 Regensburg, Germany.

The physiological effects of Endothelin 1 (vasoconstriction, positive inotropy, and stimulation of cellular growth), in normal hearts are well described. In contrast, regulation and effects of ET-1 in hearts with established left ventricular hypertrophy (LVH) are largely unknown. Rats were subjected to banding of the ascending aorta for 84 days and developed chronic LVH (LV/BW-ratio 170% of controls). ET-1 perfusion (10^{-11}-10^{-9} M) of isolated perfused hearts, at constant coronary flow and LV balloon volume, resulted in an increase of coronary perfusion pressure (CPP) in both LVH (n=8) and control groups (n=8; each 1.9-fold vs. baseline; p<0.001). In contrast, ET-1 increased LV end-diastolic pressure (LVEDP, +22±7 mmHg) only in LVH hearts (each p<0.05

vs. baseline or control. Systolic developed pressure (LVdevP) showed a slight decrease of 12 + 5 mmHg in LVH. Furthermore ET-1 perfusion resulted in a significant decrease of lactate extraction in both groups (1 mmol/g min fold vs. baseline, $p<0.05$). Administration of the phorbolester ß-PMA (10^{-7} M) mimicked ET-1 effects. In contrast, the Na-H antiporter amiloride (10^{-3} M) as well as the proteinkinase C inhibitor H7 (10^{-5} M) blocked the ET-1 mediated increase of CPP and LVEDP completely. Coadministration of nitroglycerine (10^{-4} M) also prevented the ET-1 effects on LVand lactate extraction extraction and uncovered a slight positive inotropic effect of ET-1 in both control and LVH hearts. In conclusion, hearts with established LVH are highly sensitive to ET-1 that displays PKC-mediated effects on coronary perfusion as well as systolic and diastolic function. Finally, in LVH hearts, ET-1 effects on cardiac contractility are substantially affected by consecutive coronary vasoconstriction.

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HEART PROTEIN SYNTHESIS AFTER CECAL LIGATION & PUNCTURE (CLP) IN THE RAT. M. O'Leary*, C. Ferguson, C. Hinds, J. Coakley* & V. Preedy*
St. Bartholomew's & King's College Hospitals, London, UK.

Sepsis is associated with depressed cardiac function & reduced protein synthesis (PS) in cardiac muscle has been described. We have studied changes in heart fractional rate of PS (ks, %/day), absolute rate of PS (Vs, mg protein/day), RNA activity (kRNA, Vs per mg RNA) & tissue protein content (Pc, mg) following CLP or laparotomy with starvation (LAP) in rats. 3 groups of male Wistar rats underwent CLP & 3 groups LAP with survivors sacrificed at 24, 72 or 96hrs. No food but free access to water was provided. Baseline samples were obtained at the start of the experiment (t=0). PS was measured by a flooding dose of [4-^{3}H]phenylalanine. Results (mean ± SEM) were analysed by ANOVA. Values at t=0 were (n=11): ks 7 ± 0.2, Vs 8.9 ± 0.4, kRNA 6.9 ± 0.2, Pc 126 ± 2.

		24hrs	72hrs	96hrs
n	CLP	15	9	9
	LAP	10	11	11
ks	CLP	6.4 ± 0.2*	4.6 ± 0.2#	4.6 ± 0.2#
	LAP	5.9 ± 0.2#	4.6 ± 0.1#	4.3 ± 0.3#
Vs	CLP	8.3 ± 0.3#	4.9 ± 0.3#	5.5 ± 0.2#
	LAP	7.1 ± 0.2#	4.9 ± 0.2#	5.4 ± 0.2#
kRNA	CLP	6.5 ± 0.2•	5.1 ± 0.2#	5.5 ± 0.2#
	LAP	6.3 ± 0.1°	5.4 ± 0.5#	5.4 ± 0.2#
Pc	CLP	124 ± 3	108 ± 3#	102 ± 3#
	LAP	122 ± 3	108 ± 3#	108 ± 3#

°p=0.017, •p=0.006, *p=<0.001, #p=<0.0001 compared with t=0. Cardiac muscle PS was similarly reduced by sepsis & by surgery with starvation. This suggests that the reduction in muscle PS associated with sepsis spares the heart & that cardiac muscle PS changes in sepsis & after surgery may be mainly nutritional. M O'L was supported by the JRB St. Bartholomew's Hospital, the *British Journal of Anaesthesia* & BMI Columbia Healthcare Ltd.

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SUCROSE FAILED TO PROTECT SEPTIC HEARTS FROM A CALCIUM PARADOX MEDIATED INJURY

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Sepsis produces a myocardial dysfunction, attributed in part to a decreased calcium sensitivity. The present study was designed to determine if high perfusate sucrose concentrations might protect isolated hearts of septic rats during the calcium paradox as has been observed previously with normal rat hearts. Calcium paradox experiments were conducted with Ca^{2+}-free Krebs-Henseleit medium while sucrose experiments were carried out with the same medium except that 75 mM NaCl was replaced by 150 mM sucrose during Ca^{2+} -free period. With non-septic/healthy hearts, perfusion with sucrose caused a 96% inhibition of total creatine kinase (CK) release. With surgical sham hearts, 68% of the CK release was attributed to stress associated with surgery performed. Thirty two percent of the CK release was due to the sucrose-inhibitable fraction (s.i.f.) With septic heart (from rats injected with a slurry of 200 mg of cecal material/kg), it was found that 68-92% of CK release was ascribable to surgery, 0-25% to a septic factor and 8% to the s.i.f. Thus, sucrose was responsible for inhibiting only a small fraction of CK release from septic hearts during the calcium paradox. In addition, high energy phosphate levels were decreased in septic and surgical sham hearts whereas it was unchanged in non-septic/healthy beating hearts during Ca^{2+} repletion after sucrose treatment during the preceding Ca^{2+}-free period. It is concluded that induction of sepsis made hearts more susceptible to a calcium paradox-mediated injury.

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DECREASED DIHYDROPYRIDINE RECEPTOR BINDING ([^{3}H]-PN200-110) IN CARDIAC SARCOLEMMAL VESICLES FROM LPS-TREATED GUINEA PIGS.

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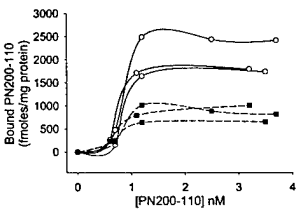
Cardiac dysfunction observed in animal models of sepsis, burn shock or LPS is associated with cytosolic calcium overload, although the source of this calcium is undetermined. Previous work from this laboratory using a guinea pig model of E. coli endotoxemia (LPS, 4mg/Kg) demonstrated reduced peak systolic Ca^{2+} (fura-2 transients) which correlated directly with reduced contractility and peak L-type Ca^{2+} current density (LPS, 3.5 ± 0.2; control 6.1 ± 0.3 pA/pF: -40 mV to 10 mV step). Reduced Ca^{2+} currents and Ca^{2+} transients were reversed by β-adrenoreceptor activation, but not Ca^{2+} channel agonist suggesting receptor activation but not receptor number was altered by endotoxemia. To determine whether cardiac L-type Ca^{2+} channel number is reduced by endotoxemia, we measured binding of [^{3}H]-PN200-110 to dihydropyridine sites on purified cardiac sarcolemmal vesicles from hearts of control and LPS treated guinea pigs. Sarcolemmal vesicles were prepared by standard sucrose gradient separation methodologies using ventricular tissue from 4 guinea pigs hearts. Each preparation provided material for 8-10 samples. Samples (300 µl, 0.04-0.08 mg protein) were incubated in [^{3}H]-PN200-110 (0.4-3.7 nM) for 60 min in the presence or absence of saturating concentrations of non-labeled nifedipine to assess nonspecific binding. Binding assays were performed in pairs with vesicles from one control and one LPS preparation. Specific binding was determined by subtracting non-specific binding for each concentration of [^{3}H]-PN200-110 for both control and LPS. These data indicate that while the K_d for binding is not different between control (open circles) and LPS (closed squares), maximal binding of [^{3}H]-PN200-110 to dihydropyridine sites is dramatically reduced in vesicles from hearts of endotoxemic guinea pigs.



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ENDOTHELIN-1 RELEASE FOLLOWS CYTOSOLIC PROTEIN WASHOUT DURING CALCIUM PARADOX AND IS INHIBITED BY SUCROSE AC Sharma[1], KJ Alden[1]*, AD Sam II[1,2], JL Ferguson[1] and A Omachi[1]*, Departments of Physiology & Biophysics[1] and Surgery[2], College of Medicine University of Illinois at Chicago, 835 South Wolcott Avenue, Chicago, IL 60612

We hypothesized that calcium paradox induction may activate the release of ET-1 and NO as well as myoglobin and creatine kinase (CK) in isolated rat heart preparation. Each excised heart was arrested in ice-cold Krebs-Henseleit (KH) medium and the cannulated heart was placed in an NMR spectrometer. During the 10 min equilibration period, heart rates stabilized at 180±8 and 192±20 beats/min in control and sucrose-treated groups, respectively. During the Ca^{2+}-free period the heart stopped beating in 2-3 min. Sucrose (150 mM)-treatment during the 10 min Ca^{2+}-free perfusion caused the heart rhythm to reappear during reperfusion at a significantly higher rate; without sucrose, there was no recovery of contractile activity. At the same time, a significant increase in fluid pressure in the perfusate in control hearts was observed in addition to significant decreases in ATP and phosphocreatine levels. Both of these changes were attenuated with sucrose. Significant peaks in myoglobin and CK release appeared at 7.5 min after initiation of reperfusion; these curves returned to basal values in 50 min. Sucrose significantly inhibited calcium paradox-induced elevation of myoglobin and CK release. A significant increase in ET-1 release was seen at 12.5 min after reperfusion, which was absent in sucrose-treated hearts. No change in the release of NOx was observed. The peaks of myoglobin and CK release rate, preceded ET-1 peak by 5 min. Thus, ET-1 release did not appear to be involved in the development of calcium paradox. However, vasoconstriction caused by ET-1 may produce reduction of nutritive flow to the heart and may subsequently exacerbate myocardial injury caused by calcium paradox. [Funded by AHA Senior fellowship (ACS) and NIH grants T32HL07692 & GM 48219 (JLF)]

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ESTROGEN'S EFFECT ON MYOCARDIAL ISCHEMIA-REPERFUSION INJURY: THE FUNCTION OF ESTROGEN RECEPTOR-α. P. Zhai*, T. Eurell*, P. Cooke*, D. Lubahn*[1], D. Gross* Uni. of IL, U-C, Urbana, IL 61801. 1 Uni. Missouri, Columbia.

Objective: To mimic the global ischemia-reperfusion injury induced during elective cardiac surgery and to study estrogen's effect under these conditions. Methods: Hearts of sham-operated or ovariectomized (OVX) adult female rats and ERKO (estrogen receptor-α knock out) and non-ERKO male mice were studied. After anesthesia, the heart was excised and dropped into 4°C, high K^+, high Mg^{++} cardioplegia solution. The mouse hearts were immersed in the same solution during ischemia. The rat hearts were perfused with cardioplegia during ischemia. After 45 min. in mice, 30 min. in rats, warm (37°C) Krebs-Henseleit, $NaHCO_3$ - buffered solution, oxygenated with 95% O_2 and 5% CO_2, was perfused into the coronary arteries using a constant pressure perfusion system. Results: Mouse: ERKOs started beating later and were more likely to develope ventricular arrhythmias, which spontaneously converted. ERKOs had more severe myocardial damage, lower coronary flow rates, reduced nitrite production, increased Ca^{++} accumulation, and worsened mitochondria respiratory function. Rat: OVX rats had impaired left ventricular function as compared to sham-operated rats in addition to other results similar to those observed in ERKOs. Conclusions: Estrogen may protect the myocardium against global ischemia-reperfusion injury via the function of α-subtype estrogen receptors.

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ABSENCE OF INDUCIBLE NITRIC OXIDE SYNTHASE ENHANCES MYOCARDIAL DAMAGE DURING EARLY REPERFUSION. B. Zingarelli, Z. Yang*, H.R. Wong*. Critical Care Medicine, Children's Hospital Medical Center, Cincinnati, OH 45229.

Controversial roles have been ascribed to nitric oxide (NO) during myocardial ischemia and reperfusion, since pharmacological inhibition of the inducible NO synthase (iNOS) may exert beneficial effects or may exacerbate the early reperfusion injury. The present report further addresses the role iNOS-derived NO in the inflammatory process of myocardial reperfusion. Myocardial injury was induced in mice genetically deficient of iNOS ($iNOS^{-/-}$) and in wild-type littermates by 1 hour occlusion of the left anterior descendant coronary artery followed by 1 hour reperfusion. In wild-type mice, ischemia and reperfusion caused myocardial injury (as evaluated by plasma levels of creatinphosphokinase, 1996±225 U/L). At immunohistochemistry evaluation an intense staining for the stress-regulated protein kinase, c-Jun NH_2 terminal kinase (JNK1), and intercellular adhesion molecule 1 (ICAM-1) was found in the necrotic tissue. The tissue damage was also associated with high plasma levels of the pro-inflammatory cytokine tumor necrosis factor (TNFα, 303±73 pg/ml) and the anti-inflammatory cytokine interleukin-10 (IL-10, 6670±1153 pg/ml). $iNOS^{-/-}$ mice subjected to myocardial ischemia and reperfusion experienced a more severe tissue injury when compared to wild-type mice (as evaluated by plasma levels of creatinphosphokinase, 2397±144 U/L, $p<0.05$). A more intense immunostaining for JNK1 and ICAM-1, was also associated with significantly higher plasma levels of IL-10 (11288±2391 pg/ml) in $iNOS^{-/-}$ mice in comparison to wild-type littermates ($p<0.05$). Plasma levels of TNFα (351±40 pg/ml) were not significantly different in comparison to the plasma levels of wild-type mice. These data demonstrate that absence of inducible nitric oxide synthase may result in increased myocardial reperfusion injury.

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FUNCTIONAL SIGNIFICANCE OF ENDOTHELIN-B RECEPTOR EXPRESSION IN ENDOTOXEMIA. M. Bauer, I. Bauer*, N. Sonin*, N. Kresge*, R. Baveja*, D. Harding*, Y. Yokoyama*, J.X. Zhang*, M.G. Clemens. Univ. North Carolina at Charlotte, Charlotte, NC 28223

Endothelins are important regulators of liver blood flow. Response to ET-1 is increased by endotoxin (1mg/kg, LPS) but the role of ET_B receptors is not known. We tested the functional significance of endothelin B (ET_B) receptors in regulating sinusoidal as well as extra-sinusoidal micro-hemodynamics in livers of normal and LPS primed rats. Infusion of the ET_B agonist IRL 1620 increased portal pressure of isolated perfused liver dose dependently; this effect was greatly potentiated by pretreatment with L-NAME. *In vivo* video microscopy during IRL1620 revealed disruption of sinusoidal flow patterns (areas of reverse flow and shutdown) without causing sinusoid constriction; however, significant sinusoid constriction colocalizing with hepatic stellate cells was observed with IRL1620 after L-NAME pretreatment. LPS priming resulted in a significant increase in ET_B receptor transcripts (RT-PCR) and a shift to predominantly ET_B

available receptors (competitive binding). In the isolated perfused liver, increased ET_B expression did not affect the peak portal pressor response but prolonged the response. *In vivo*, LPS priming resulted in maintained response to IRL 1620 while the response to the α_1 adrenergic agonist was substantially blunted. These results suggest that ET_B receptors can modulate the portal and sinusoidal response to ETs in part via activation of nitric oxide synthase (NOS) activity. We propose that ET_{B1}-induced NOS activity attenuates ET_{B2}- mediated portal pressor response and stellate cell constriction and protects against hepatocellular damage. Induction of the ET_B gene appears to serve to prevent tachyphylaxis as is observed with α_1 adrenergic receptors. Supported by NIH DK38201 and the Alexander von Humboldt Foundation.

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MICROHETEROGENEITY OF LIVER PO_2 IN RESPONSE TO ENDOTHELIN-1: EXACERBATION BY LPS PRIMING. R Baveja*, J Zhang*, Y Yokoyama*, N Sonin*, MG Clemens. University of North Carolina at Charlotte, NC 28223.

Endothelin-1 is a potent vasoactive peptide that acts at sinusoidal and extrasinusoidal sites in liver. ET-1 sensitivity increases in LPS primed animals and impairs liver microcirculation in these animals. We hypothesized that ET-1 causes a microheterogeneous distribution of liver O_2 which is further exacerbated in LPS primed animals. Rats were studied 24 hrs after LPS (1 mg/kg, ip). Surface PO_2 was determined using a recently developed technology of O_2 mapping. Previous experiments using ET-1 and phenylephrine showed a microheterogeneous distribution of PO_2 with ET-1 as a function of time with areas of high fluorescent intensity corresponding to low PO_2 and corresponding to reduced pyridine nucleotides redox potential. The baseline portal pressure was higher in LPS primed animals ($p<0.05$) and increased to similar extent as sham animals after 10 min infusion of ET-1. There was no significant difference in baseline mean arterial pressure and no significant systemic response to ET-1 in either group. The mean fluorescent intensity (higher intensity= less O_2) increased from baseline levels 33.8±9 to 46.8 ±8.3 in sham; 42.3±9.1 to 69±6.5 in LPS ($p<0.01$ sham vs LPS), at end of infusion of ET-1 for 10 min. This indicates hypoxia without ET-1 infusion in LPS primed rats which is exacerbated by ET-1. Frequency distribution analysis showed a shift in the mode from lower intensity (41-50, higher PO_2) to areas with higher fluorescent intensity ranges (61-70 and 71-80, lower PO_2) indicating areas with shut down in perfusion in LPS treated animals. We propose that LPS exacerbates the microheterogeneity in liver surface PO_2 response to ET-1 resulting in areas of focal hypoxia. Supported by NIH DK38201

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DIASPIRIN CROSS-LINKED HEMOGLOBIN (DCLHb) RESTORES PANCREATIC MICROCIRCULATORY FAILURE AFTER HEMORRHAGIC SHOCK. E. v. Dobschuetz*, T. Hoffmann*, K. Messmer. Institute for Surgical Research, Ludwig-Maximilians-University, Munich, Germany. During clinical trials of the oxygen carrier DCLHb an elevation of amylase activity and clinical signs of acute pancreatitis occurred in some patients. Since DCLHb has vasoconstrictive properties, this elevation might have been caused by microcirculatory disturbances, which induced ischemic damage in the pancreas. We, therefore, investigated the effects of DCLHb on the microcirculation of the pancreas predamaged by hemorrhagic shock. Hemorrhagic shock was induced by withdrawal of blood from the carotid artery and maintenance of mean arterial pressure of 40 mmHg for one hour in anaesthetised male rats. Rats were resuscitated by either (n=7 per group) (a) 6% Hydroxyethylstarch, (b) DCLHb, or (c) whole blood in amounts equivalent to shed volume. Sham animals without shock induction served as control groups. The length of red blood cell perfused capillaries per pancreatic surface area (FCD, cm^{-1}) and the number of adherent leukocytes in postcapillary venules (ad. leuk., cells/mm^2) were measured by intravital epifluorescence microscopy two hours after infusion (2h p. res.). Thiobarbituric acid reactive substances (TBRS, nmol/g) as a parameter of lipid-peroxidative damage in pancreatic tissue were determined at the end of the observation time.

2h p.res.	sham	HAES 6%	DCLHb	wh. blood
FCD	366 ± 11	241 ± 13*	294 ± 17	306 ± 11
ad. leuk	149 ± 41	615 ± 107*	369 ± 56	510 ± 157
TBRS	0	0.3 ± 0.2	4.8 ± 0.7*	3.7 ± 0.7*

Values are mean ± SEM. *P<0.05 vs. sham, Dunn's method. Resuscitation with a non oxygen carrying solution could not reverse microcirculatory perfusion failure. DCLHb restored microcirculation after hemorrhagic shock and increased pancreatic lipidperoxidation as whole blood. Thus DCLHb showed a beneficial effect on the pancreatic microcirculation, indicating it is as a valuable resuscitation fluid.

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THE HEMODYNAMIC AND HEPATIC MICROCIRCULATIONAL CHANGES OF SEPTIC RATS TREATED WITH DIFFERENT NOS INHIBITORS. T.L. Hwang, C.C. Yeh*, Department of Surgery, Chang Gung Memorial Hospital, Taipei, Taiwan.

Severe septic shock may produce hypotension which is due to the vasodilated effect of nitric oxide. The effects of different Nitric oxide synthase (NOS) inhibitors on hemodynamic and hepatic microcirculation of septic rats were studied. Male Sprague-Dweley rats were divided into three groups. They were cannulated with femoral arterial, femoral venous and jugula venous catheters. Cardiac output was measured with thermodilutional method and liver sinusoidal microcirculation was measured with Laser Doppler Flowmetry. Group A rats (n = 6) were injected with lipopolysaccharide (LPS) (50 mg/kg BW) and L-NAME (5 mg/kg BW), group B rats (n = 6) were injected with same dose of LPS and aminoguanidine (400 μmole/kg BW), group C rats (n = 6) were injected with same dose of LPS and normal saline as control. The cardiac outputs, stroke volumes, heart rates, blood pressures, microcirculational flux of liver in three groups were measured and compared at 0, 20, 40, 60 and 80 minutes after injection. The cardiac output, stroke volume significantly decreased though the blood pressure increased in Group A, these changes were less severe in group B. Hepatic microcirculation showed mild decreased in group A, but increased in group B.

	Gp. A	Gp. B	Gp. C
Cardiac output (ml/min)	63.7±7.3*	104.8±10.3	93.2±10.0
Stroke volume (ml/min)	168.7±35.8*	285.7±26.9	242.2±21.4
Systolic pressure (mmHg)	147.3±17.7*	132.3±8.1#	117.2±14.6
Diastolic pressure	102.0±24.0*	76.5±7.7	67.5±10.3
Sinusoidal flux	218.8±26.4	348.7±82.0#	253.2±31.6

*, #: $p < 0.05$, vs Gp. C

We concluded that the aminoguanidine is better to prevent the hypotensive effect during severe sepsis, which can also better maintain cardiac output and hepatic microcirculation than L-NAME.

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ZONAL HETEROGENEITY OF CD95-MEDIATED HEPATIC MICROVASCULAR PERFUSION FAILURE - ROLE OF CASPASES L. Mica, G.A. Wanner, *H. Hentze, O. Trentz, W. Ertel. Division of Trauma Surgery, University Hospital of Zurich, Zurich, Switzerland; *Department of Biochemical Pharmacology, University of Konstanz, Konstanz, Germany.

The effect of agonistic CD95 antibodies (aCD95) on sinusoidal perfusion and a potential protection by caspase inhibition was studied in a murine model using intravital fluorescence microscopy. C3H/HeN mice were intravenously administered aCD95 (10 μg/mouse) or unspecific IgG (control) in the presence or absence of the caspase inhibitor z-VAD-fmk. Treatment of animals with aCD95 resulted in a significant ($p<0.01$) increase of caspase-3-like activity in liver tissue associated with severe ($p<0.01$) deterioration of sinusoidal perfusion characterized by 36.6 ± 4.8 % non-perfused sinusoids after 2 hours. The administration of z-VAD-fmk after aCD95 application completely blocked caspase activity in liver tissue and attenuated ($p<0.01$) sinusoidal perfusion at 2 hours (17.3 ± 3.8 % non-perfused sinusoids). Analysis of zonal distribution of sinusoidal perfusion failure demonstrated that the effect of aCD95 was most pronounced in the midzonal and in the pericentral region (38.3 ± 4.4 % and 49.1 % ± 4.2 of non-perfused sinusoids). While z-VAD-fmk failed to inhibit perfusion failure of periportal sinusoids, perfusion was markedly ($p<0.01$) improved (19.3 ± 3.5 % non-perfused sinusoids) in the midzonal region and almost completely restored (12.7 ± 4.8 % non-perfused sinusoids) in the pericentral region ($p<0.01$). These data demonstrate that CD95-mediated microvascular damage is in part independent of caspase activation, because z-VAD-fmk though completely blocking caspase activity in the liver was ineffective on sinusoidal perfusion failure in the periportal zone and did not fully prevent hypoperfusion of midzonal sinusoids. Therefore, caspase inhibition may not completely prevent CD95-mediated liver injury.

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THE EFFECT OF INTESTINAL ISCHEMIA/REPERFUSION (II/R) ON LEUKOCYTE/ENDOTHELIAL INTERACTION. S.I.Myers, M. Badellino*, L. Bartula*, A.R. Seelig*, R. Milner*, X.Ni* and R. Tuma*. Temple Univ., Phila. PA 19140.

This study examines the hypothesis that II/R enhances leukocyte/endothelial cell interactions in a remote organ. Chronic microvascular skin-fold chambers were implanted in female nude mice (20-25g). After 2 days, the mice were anesthetized and a laparotomy was performed. Intestinal ischemia (30 minute occlusion of superior mesenteric artery) or sham operation was performed. A jugular catheter was implemented to inject rhodamine dye for the *in vivo* fluorescent labeling of leukocytes. Readings were made at baseline (pre-injury) and at specific times of reperfusion (5min, 15min, 30min and 45 min). Microvascular examination via epiillumination fluorescent microscopy (EFM) was used to visualize the leukocytes "rolling" along a specific point of each arteriole vessel over a period of 30 seconds. Data is expressed as the average number of leukocytes "rolling"/min. Data is Mean±S.E.M. (N=6,*-$p<0.001$ by repeated measures ANOVA).

Reperfusion Time	SHAM	II/R
5 min	0.68±0.33	4.19±1.22*
15 min	0.77±0.11	2.74±0.31
30 min	0.68±0.20	2.76±0.43
45 min	0.33±0.18	2.08±0.45

The results demonstrate that intestinal ischemia followed by reperfusion for 5 minutes increases the "rolling" of leukocytes along the endothelial cells of arterioles. Increased leukocyte rolling continued to be several fold higher than sham at the later time points yet these time periods did not reach statistical significance. These data suggest two conclusions. First, II/R increased leukocyte/endothelial cell interaction in a remote organ (skin fold) showing that this injury can cause alterations in microvascular function. Second, the use of the chronic skinfold chamber in the mouse is an ideal method for examining altered leukocyte function following intestinal ischemia.

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VENTING OF CEREBROSPINAL FLUID VIA CERVICAL LYMPHATIC VESSELS FOLLOWING AN INCREASE IN INTRACRANIAL PRESSURE. I. Silver,* B. Li,* J. Szalai,* and M. Johnston Trauma Research and Department of Laboratory Medicine and Pathobiology, Sunnybrook and Women's College Health Sciences Centre, University of Toronto, M4N 3M5.

Previous studies from our group have demonstrated that in resting states, about one-half of the total volume of cerebrospinal fluid (CSF) removed from the cranial vault transports along the arachnoid sheaths of the olfactory nerves, through the cribriform plate into the cervical lymphatic vessels (Am. J. Physiol. 272: R1613, 1998; 274: R88, 1998). In this study we tested the hypothesis that an elevation of intracranial pressure (ICP) results in an increase in lymph flow rates in anesthetized sheep. Catheters were inserted into both lateral ventricles, the cisterna magna, cervical lymphatics and jugular vein. A ventriculo-cisternal perfusion system was employed to regulate CSF pressures and ICP was controlled by adjusting the height of the inflow reservoir and the cisterna magna outflow catheter appropriately. Cervical lymph flow rates increased incrementally as ICP was elevated (10, 30, 50 and 70 cm H_2O ($p<0.0001$). On average, cervical flow rates increased 4 fold between 10 and 70 cm H_2O. Additionally, intralymphatic pressure (measured with a Millar transducer catheter) increased as ICP was raised. Central venous pressure was unchanged. No changes were observed in mesenteric lymph flow rates which were measured concurrently. These results suggested the existence of an hydraulic connection between the CSF and cervical lymph compartments. Cervical lymphatics provide an important CSF venting mechanism and we estimate that ~ 75% of the total lymph in cervical vessels was derived from CSF at 70 cm H_2O ICP. Supported by the Medical Research Council of Canada.

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DOSE DEPENDENT INDUCTION OF EXPRESSION OF HEPATIC VASCULAR STRESS GENES BY LPS . NV Sonin*, I Bauer*, M Bauer, JX Zhang*, and MG Clemens. Dept of Biology, University of NC at Charlotte, Charlotte, NC 28223-0001

Endotoxemia, induced by moderate doses of LPS leads to an upregulation of both constrictors and dilators in the rat liver and increased responsiveness to the vasoconstrictor endothelin 1. Time course and dose response of these changes have not yet been studied. We used a semiquantitative RT-PCR to estimate relative changes in mRNA level for ET-1, its receptor subtypes (ET_A and ET_B), and iNOS. Saline (sham) or LPS at concentration 0.5, 1.0, 2.0, 5.0 mg/kg b.w. was injected ip, and liver samples were taken at 6 and 24 hrs after the injection. RT-PCR was performed on standardized RNA samples and final PCR-products were analyzed by densitometry. At 6 hrs LPS markedly increased mRNA level for ET-1, ET_B , and iNOS

in a dose-dependent manner. Expression of ET_A mRNA was downregulated at low conc. of LPS - 0.5 mg/ml (Table 1). At 24 hrs after LPS injection ET-1, ET_B, and iNOS mRNA were slightly elevated (data not shown).

Relative levels of mRNA in control and at 6 hrs after LPS induction

(arbitrary densitometry units)

LPS dose	0	0.5	1.0	2.0	5.0
ET-1	0.6 ± 0.1	1.7± 0.1	2.5± 0.2	3.1± 0.1	1.7±0.9
ET_A	7.3± 0.8	4.8± 0.5	7.8± 0.8	6.9± 0.7	6.0±0.8
ET_B	5.5± 0.4	9.3± 0.9	9.3± 0.9	8.8± 1.1	9.3±0.5
iNOS	0.7 ± 0.7	5.1± 0.4	8.8± 0.7	8.4± 0.4	5.3±0.5

These data demonstrate a complex dose-dependent change in expression of vasoconstictor and dilator genes and divergent expression of ET-1 receptors. Those changes may explain heterogeneous responses of hepatic microcirculation in endotoxemia.

Supported by NIH grant DK38201.

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EXPRESSION AND LOCALIZATION OF ENDOTHELIN B (ET_B) RECEPTORS IN LPS PRIMING. Yukihiro Yokoyama*, R Baveja*, N Sonin*, K Nakanishi*, JX Zhang, and MG Clemens Dept of Biology, Univ of North Carolina at Charlotte, NC 28223

Endothelins are important regulators of liver blood flow and may contribute to altered hemodynamics in shock. Therefore, we studied the changes in endothelin (ET) receptor subtype expression in endotoxin (1mg/kg, LPS) primed rat liver using *in vitro* and *in situ* receptor binding. 24 hours after LPS injection livers were flushed for 30 minutes to minimize endogenous binding resulting from high levels of *in vivo* endothelin expression during endotoxin priming. Livers were homogenized for *in vitro* receptor binding assay using 100pM [^{125}I]-ET-1 with varying concentration of unlabeled ET-1. Dissociation constant (Kd) and total number of receptors (Bmax) were calculated by Scatchard analysis. The proportion of ET_A and ET_B receptor were determined using BQ-610 (ET_A antagonist) and IRL-1620 (ET_B agonist). In addition, *in situ* receptor binding and autoradiography were performed to determine the acinar distribution of ET receptor subtypes. LPS-pretreated group showed significant increases in Bmax for ET-1 (51.9 ± 6.3, LPS vs 28.9 ± 4.0, control, fmol/mg protein, $P<0.0005$) This was primarily due to an increase in ET_B receptor proportion (78.5% over control, $P<0.001$). *In situ* autoradiography showed ET_B receptors to be concentrated in zone1 and zone3. In contrast, ET_A receptor distribution was concentrated around the portal triad and, to a lesser extent, in the sinusoids. Acinar distribution of receptor subtypes was not altered by LPS. Our results indicate that the primary ET receptor subtype upregulated by LPS (i.e. ET_B) is distributed near the outflow of the sinusoids. This distribution may contribute to the disruption of sinusoidal flow observed during inflammatory states such as LPS priming. Supported by NIH DK38201.

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POLYNITROXYLATION REDUCES THE PRESSOR ACTIVITY OF αα-CROSSLINKED HEMOGLOBIN: HEMORRHAGIC SHOCK RESUSCITATION IN THE RAT. P. Buehler, S. Mehendale, H. Wang, A. Gulati, L. Ma*, C.E. Trimble* and C.J.C. Hsia*. University of Illinois, Chicago, IL 60612 and *SynZyme Technologies LLC, Irvine, CA 92618

Objective: Some potential hemoglobin-based oxygen carriers (HBOCs) have adverse hemodynamic effects such as hypertension and compromise of regional blood flows, possibly due to binding and/or oxidation of nitric oxide. We hypothesize that adding antioxidant activity to HBOC may reduce these effects and improve safety and efficacy. To test this hypothesis we labeled an HBOC with nitroxide, which can mimic antioxidant enzymes. Methods: Polynitroxyl hemoglobin (PNH) was prepared by labeling αα-crosslinked Hb (ααHb) with 16 equivalents of nitroxide. In anesthetized rats, the following were measured after 30 min hemorrhagic shock and 60 min full-volume resuscitation with PNH, ααHb, or saline: mean arterial pressure (MAP), heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR), base-deficit (BD) and regional blood flows (radioactive microsphere method). Results: Survival times were: saline, 109 ± 7 min; ααHb, 378 ± 67 min; PNH, 483 ± 67. The table shows physiologic parameters measured 60 min post-resuscitation (% change from hemorrhage levels):

	MAP	SV	HR	CO	TPR	BD
Saline	+5	-29	-20	+5	+4	-11
ααHb	+185	+130	+10	+148	+52	+47
PNH	+151	+219	+11	+252	-20	+54

Compared with ααHb, PNH decreased vascular resistance in brain, gut, and skeletal muscle. Conclusions: PNH displayed less pressor activity than ααHb. Furthermore, by decreasing vascular resistance, particularly in gut and skeletal muscle, PNH may enhance perfusion, reduce secondary injury, and improve outcome following shock. (Supported by the Office of Naval Research, N00014-98-C-0140.)

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EFFECT OF PICROLIV ON THE EXPRESSION OF INSULIN-LIKE GROWTH FACTOR (IGF)-I, IGF-II AND IGF-I RECEPTOR IN RATS EXPOSED TO HYPOXIA. J. P. Gaddipati*, H. Mani*, S. K. Sharma*[1], D. K. Kulshreshtha*[1] and R. K. Maheshwari*, (Spon: P. Rhee). Center for Combat Casualty Care and Life Sustainment Research, Department of Pathology, USUHS, Bethesda, MD 20814 and [1]Central Drug Research Institute, Lucknow, India.

The insulin-like growth factors (IGFs), IGF-I and IGF-II play important roles in normal growth and differentiation. In recent studies IGFs have been implicated in tissue repair and regeneration after hypoxic-ischemic injury. The growth effects of these genes are exerted primarily through IGF-I receptor (IGF-IR). We have earlier shown that picroliv, obtained from the roots of *Picrorhiza kurrooa*, reduces cellular damage caused by hypoxia *in vitro*. In the present study, the role of IGF-I, IGF-II and IGF-IR in hypoxia and the ability of picroliv to modify their expression was studied *in vivo*. Male Sprague Dawley rats placed in 10% oxygen for four days were sacrificed and the expression of IGF-I, IGF-II and IGF-IR was determined by immunohistochemistry and *in situ* hybridization in brain, liver and lung. One group of animals was pretreated with picroliv. IGF-I and IGF-IR were expressed in distinct regions of the brain but not in liver or lung. IGF-1 was mainly expressed in the hippocampus whereas IGF-IR was expressed in cerebellum and cortex. A significant reduction in the mRNA level of these genes was observed in response to hypoxia. Pretreatment with picroliv prevented such down-regulation. The mRNA level of IGF-II was constitutively expressed and did not alter by hypoxia. Thus, IGF-I and IGF-IR are differentially expressed and appear to play important role in hypoxia. Modulation of their expression by picroliv, a novel pharmacological agent, could benefit in similar clinical settings. (Supported by ONR Grant G174HV and G174GV).

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COLD STORAGE HAS A MODEST EFFECT ON PHENYLEPHRINE-INDUCED CONTRACTION OF AORTA. Patrick D Harris, Jing Hu*, and Touichi Kawabe*. Ctr Applied Microcirc Res & Dept Physiology Univ Louisville, KY 40292

Harvested vessels are often refrigerated for 24 hours prior to use in tissue bath studies to determine vascular reactivity to vasoconstrictors and vasodilators in pathophysiologic states such as shock. Alpha-1 adrenergic receptor-mediated contraction of fresh aorta is blunted by endothelial release of vasodilators over time. Our study asked: Is aortic-ring contraction to Phenylephrine (PE), a selective alpha-1 adrenergic receptor agonist, affected by 24-hour cold storage? Aortic segments were harvested as four vessel rings from each of 6 rats. Half of these rings were used in 60-75 minutes (1H) and the other half were refrigerated in PSS for 24 hours (24H). Rings were put on 2 and 6gm preloads (PL); stress-relaxed for 45 minutes; contracted with 5 doses of PE (0.03-3uM), and relaxed with ACH (1uM). This PE curve (cv) + ACH was repeated 5 times over 5 hours. The maximum contraction (Fmax) of aorta to PE in cv-1 was larger at 24 H than at 1H. The Fmax of aorta to PE in cv-1 was larger at 6gm than at 2gm PL at both 1H and 24H. Aorta reactivity (pD2) to PE in cv-1 was greater at 24H over that at 1H. The pD2 for aorta contraction to PE in cv-1 was the same at 6 and 2gm PL for both 1H and 24H tissues. The Fmax of aorta to PE at 2gm PL decreased over 5 hours at both 1H and 24H. The pD2 of aorta to PE at 2gm PL decreased over 5 hours at both 1H and 24H. 24-hour storage reduced ACH-induced relaxation of aorta from 77% to 65%. 24-hour cold storage had a modest effect on aorta to increase alpha-1 adrenergic receptor-mediated contraction. This change in PE-induced contraction appears to be related to a modest effect of 24-hour cold storage on endothelial cells to decrease endothelial-dependent relaxation of the aorta. (Funded: CAMR, UofL Graduate School)

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COLD STORAGE SIGNIFICANTLY ALTERS PHENYLEPHRINE-INDUCED CONTRACTION OF CAROTID ARTERY. Jing Hu*, Patrick D Harris, and Touichi Kawabe*. Dept Physiology & Ctr Applied Microcirc Res Univ Louisville, KY 40292

Phenylephrine (PE) contractions differ between the elastic aorta and muscular carotid arteries, depending on preload (PL) and duration of PE exposure. Our study asked: is PE-induced contraction of muscular carotid arteries affected more or less than aorta by 24-hour cold storage. Both right (RCA) and left (LCA) carotid arteries were harvested as 4 vessel rings from each of 6 rats. Half of these rings were used in 60-75 minutes (1H) and the other half were refrigerated in PSS for 24 hours (24H). Rings were put on 2 and 6gm PL; stress-relaxed for 45 minutes; contracted with 5 doses of PE (0.03-3uM), and relaxed with ACH (1uM). This PE curve (cv) + ACH was repeated 5 times over 5 hours. The maximum contraction (Fmax) of both RCA and LCA to PE in cv-1 was larger at 24 H over that at 1H. The Fmax to PE in cv-1 was larger at 6gm than at 2gm PL for both RCA and LCA at 1H; but not for either vessel at 24H. Vessel reactivity (pD2) to PE in cv-1 was greater at 24H over that at 1H for both RCA and LCA. The pD2 to PE in cv-1 was greater at 6gm than at 2gm PL for both RCA and LCA at both 1H and 24H. The Fmax to PE at 2gm PL increased over 5 hours for both RCA and LCA at 1H but not at 24H. The pD2 to PE at 2gm PL increased over 5 hours for both RCA and LCA at 1H but much less so at 24H. 24-hour storage greatly reduced ACH-induced relaxation from 83% to 26% for RCA and from 87% to 17% for LCA. 24-hour cold storage gave a large increase in alpha-1 adrenergic receptor-mediated contraction of muscular carotid arteries. This appears to be related to a large effect of 24-hour cold storage to decrease endothelial-dependent relaxation of muscular arteries. There is a much greater cold-storage effect on the muscular carotid artery than on the elastic aorta. (Funded: CAMR, UofL Grad. Sch)

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***ESCHERICHIA COLI* LPS-PRIMED RAT BRAIN MICROGLIA SUPEROXIDE AND THROMBOXANE B_2 GENERATION IS INHIBITED BY THE MARINE PSEUDOPTEROSINS.** A.M.S. Mayer[1], S. Oh[1]*, W. Fenical[2]* and R.S. Jacobs[3]* (1) Midwestern Univ., Downers Grove, IL 60515, (2) Univ. of California San Diego, CA 92093 and (3) Univ. of California Santa Barbara, CA 93106.

Reduction of brain microglia (BMΦ) O_2^- generation has been proposed as a treatment strategy for septic shock and CNS pathologies. We have shown the LPS-primed BMΦ is a convenient *in vitro* model to investigate antiinflammatory marine natural products (**SHOCK** 7:49, 1997). The marine anti-inflammatory Pseudopterosins (PS) potently inhibit leukocyte eicosanoid production (**Life Sciences** 62:401, 1998). The purpose of this investigation was to study the effect of 4 PS analogs: PSA, PSE, PSA methyl-ether (PSAM) and PSC on phorbol ester-stimulated thromboxane B_2 (TXB_2) and superoxide anion (O_2^-) generation from *E. coli* LPS (0.3ng/ml)-activated BMΦ. O_2^- was determined by SOD-inhibitable reduction of ferricytochrome C and TXB_2 by EIA. The results of 3 independent experiments were the following.

PS	O_2^-[1] $IC_{50}\mu M$	TXB_2[2] $IC_{50}\mu M$	LDH[3] $IC_{50}\mu M$
A	1.1	1.3	1.3
M	1.3	1.1	2.4
C	1.5	1.2	2.2
E	2	1.9	7.8

[1] 50% of control O_2^-, [2] TXB_2 and [3] LDH release.

The fact that the PS analogs tested concomitantly reduced both BMΦ O_2^- and TXB_2 supports the hypothesis that PS inhibit eicosanoid generation by a cyclooxygenase (COX)-independent mechanism, possibly distal to arachidonic acid release, because we have recently reported that BMΦ O_2^- generation is not affected by COX inhibitors (*Soc. Neurosci. Abs.t.* 23:1223, 1997). *Supported by US Dept. Commerce grant NA66RG0477 (Project R/MP-73) and Midwestern University.*

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PREVENTION OF RENAL ISCHEMIA-REPERFUSION INJURY IN RATS : ANTIOXIDANT ROLE OF PICROLIV. P. Seth*, A. K Singh*, R. Kumari*, S. Madhavan* H. Mani*, S. K. Sharma[1], D. K. Kulshreshtha[1] and R. K. Maheshwari*, (Spon: P. Rhee) *Centre for Combat Casualty Care and Life Sustainment Research, Department of Pathology, USUHS, Bethesda, MD-20814 and [1]Central Drug Research Institute, Lucknow, India.

Picroliv, is a potent antioxidant, extracted from the roots and rhizome of *Picrorhiza kurrooa*. It has been shown to impart significant hepatoprotective activities, by modulation of free radical induced lipid peroxidation. Involvement of oxygen free radicals in ischemia reperfusion injury (IRI) is based on measurement of increased lipid peroxidation products. Lipid peroxidation is a critical pathway of reactive oxygen species inducing tissue injury in post-ischemic acute renal failure. The efficacy of picroliv was assessed in an *in vivo* model of renal IRI. Male Sprague Dawley rats were fed with 12mg/kg dose of picroliv once daily for 7 days prior to renal IRI, which was induced by clamping of renal artery for 60 mins of the left kidney, while right kidney served as internal control. Renal

blood flow was restored by releasing the microaneurysm clips to allow reperfusion and animals were sacrificed following varying time of reperfusion. Increased lipid peroxidation and apoptotic cell number reflected the oxidative damage following renal IRI. Biochemical analysis of kidney samples were performed to study the reduced glutathione (GSH) levels and activities of glutathione peroxidase (GPx) and glutathione reductase (GR) enzymes, the two important enzymes of the GSH redox cycle. Picroliv pretreated rats exhibited lower lipid peroxidation, better antioxidant status, and reduced apoptosis, indicating better viability of renal cells. Immunohistochemical studies revealed that picroliv pretreatment attenuated expression of ICAM-1 and CD-18 in glomerular region. Overall, picroliv pretreatment appears to protect rat kidneys from ischemia-reperfusion injury, perhaps by modulation of free radical damage and adhesion molecules. (Supported by ONR grant # G174HV)

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PREVENTION OF CELL DEATH FOLLOWING ISCHEMIA-REPERFUSION INJURY BY PICROLIV.
A. K. Singh*, P. Seth*, R. Kumari*, H.Mani*, D.K. Kulshreshtha[1]*, S.K. Sharma[1]*, and R.K. Maheshwari*, (Spon: P. Rhee). Center for Combat Casualty & Life Sustainment Research, Department of Pathology, USUHS, Bethesda,MD, 20814; [1]Central Drug Research Institute, Lucknow, India.

Cell death following ischemia-reperfusion injury is a major concern in clinical issues such as organ transplantation and trauma. The need to identify agents with potential for preventing such damage has assumed great importance. We have evaluated the efficacy of picroliv, a product derived from the plant *Picrorhiza kurrooa*, in protecting against hepatic ischemia-reperfusion injury *in vivo*. Picroliv was fed to male Sprague Dawley rats in a dose of 12 mg/kg once daily by oral gavage for seven days prior to hepatic ischemia which was induced by occluding the hepatic pedicel with a microaneurysm clip for 30 min. Reperfusion was allowed thereafter for varying periods of 15-120 min. We have studied the effect of picroliv against apoptosis and proliferative capacity (by staining for proliferating cell nuclear antigen (PCNA) in the hepatocytes. The number of apoptotic cells increased with increasing duration of reperfusion. Hepatocyte apoptosis was reduced following picroliv treatment. PCNA immunoreactivity was also greater in this group. The ratio of PCNA positive cells to TUNEL positive cells was calculated and was found to be significantly more in picroliv fed animals thereby indirectly indicating greater vitality and viability of hepatocytes. Picroliv pretreatment also resulted in lower levels of lipid peroxidation products, higher cellular SOD thereby suggesting it to be a promising agent for ameliorating injury following ischemia-reperfusion.(Supported by ONR grant # G174HV).

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COLONIC AND SYSTEMIC HEMODYNAMIC ALTERATIONS DURING IV INFUSION OF ATP-$MgCl_2$ IN HORSES.
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The purpose of this study was to determine the effects of intravenous (IV) infusion of adenosine triphosphate-magnesium chloride (ATP-$MgCl_2$) on local arterial blood flow, arterial and venous pressure, and vascular resistance in the large colon, and selected systemic hemodynamic variables in anesthetized horses. Twelve adult horses were divided into 2 equal treatment groups. Group 1 (control) received saline and group 2 (treated) received ATP-$MgCl_2$ IV beginning at a rate of 0.1 mg ATP/kg of body weight/min. The infusion was increased by 0.1 mg/kg/min increments at 10-minute intervals until a rate of 1.0 mg/kg/min was achieved. Selected variables measured (or calculated) before, during, and after the infusion included cardiac output (CO), heart rate (HR), mean systemic arterial pressure (MAP), systemic vascular resistance (SVR), mean colonic arterial and venous pressure (CAP and CVP), mean colonic arterial blood flow (CBF), and colonic vascular resistance (CVR). There were no significant differences between groups for any variable pre-infusion. There were no significant differences across time for any variable in group 1. CO was significantly increased in group 2 only at 0.7 mg/kg/min. There was no significant difference in HR across time. MAP significantly decreased, starting at 0.2 mg/kg/min, and remained below baseline values throughout the study. SVR was significantly decreased from 0.4-1.0 mg/kg/min. CAP significantly decreased, starting at 0.2 mg/kg/min, and remained below baseline values throughout the study. No consistent significant changes were observed in CVP. CBF was significantly decreased from 0.4 mg/kg/min until 2-min post-infusion. CVR was significantly decreased from 0.3 mg/kg/min to 15-min post-infusion. IV infusion of ATP-$MgCl_2$ resulted in a significant decrease in CVR, primarily via colonic arterial vasodilatation. The efficacy of this agent in the treatment of equine ischemic bowel disease warrants further investigation.

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POLYNITROXYL-ALBUMIN (PNA) PLUS TEMPOL INHIBIT LUNG INJURY SECONDARY TO INTESTINAL ISCHEMIA/REPERFUSION
S. Zhang, D. Carden, L. Ma*, C.E. Trimble*, and C.J.C. Hsia*. LSU Medical Center, Shreveport LA 71130 and *SynZyme Technologies LLC, Irvine CA 92618

Objective: Gut ischemia (I) and reperfusion (R) are associated with secondary lung injury. Reactive oxygen species (ROS) are central to the primary gut injury, and perhaps also the secondary lung injury. Nitroxide-labeled macromolecules, acting alone or in conjunction with the free nitroxide Tempol, can inhibit ROS toxicity. This study asked whether nitroxides can inhibit the lung injury elicited by gut I/R.
Methods: PNA (200 mg/ml) was prepared by labeling human serum albumin (HSA) with nitroxide. Rats were subjected to 2 hr I (superior mesenteric artery occlusion) and 20 min R. Treatments: PNA; PNA plus Tempol 10 mg/ml; HSA; or saline. Dose: 4.5 ml/kg before I, then 5.5 ml/kg infused during I. After R, rats were exsanguinated and the blood was used to perfuse isolated rat lungs. Capillary filtration coefficient (Kf,c) and tissue myeloperoxidase (MPO) activity were measured in the isolated lungs.
Results (*$p<.05$ vs. control):

Treatment	Kf,c (ml/min/ cm H_2O/100g)	MPO activity (units/g wet wt)
Control (no I/R)	0.114 +/- 0.013	3.8 +/- 0.25
I/R + saline	0.268 +/- 0.03*	9.25 +/- 0.15*
I/R + HSA	0.336 +/- 0.112*	6.75 +/- 1.8*
I/R + PNA	0.360 +/- 0.05*	10.15 +/- 1.7*
I/R+PNA +Tempol	0.082 +/- 0.024	9.25 +/- 0.75*

Conclusions: PNA+Tempol administered during gut I/R prevented secondary microvascular injury to the lung. We have previously shown that PNA alone reduces local leukocyte adhesion and emigration following 20 min intestinal I and 30 min R, suggesting that this macromolecule attenuates reperfusion injury in the gut (Free Radical Biol Med 25: 153-159, 1998). The present results show that the small, membrane-permeant nitroxide Tempol is required in addition to PNA to prevent the pulmonary microvascular injury elicited by prolonged intestinal I/R.
(Supported by NIH, NIDDK 2 PO1 DK 4378506)

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ANISODAMINE ATTENTUATED TNF-α MEDIATED APOPTOSIS IN ENDOTHELIAL CELLS THROUGH A NF-κ B-DEPENDENT MECHANISM. Lin Zhong*, Weimin Xiao*, Meidong Lui*, Jialu You* and Zhengyao Luo*, (Spon: Shilin He). Dept. Of Pathophysiology, Hunan Medical University, Changsha, Hunan, 410078 P.R.China.

Anisodamine, a Chinese tranditional medicine herb, has been effectively used for treatment of adult respiratory distress syndrome, but little is known about its mechanism. Previous study in our laboratory demonstrated that anisodamine could attenuate bovine pulmonary artery endothelial cell (BPAEC) injury induced by endotoxin. Recently we reported that TNF-α induces apoptosis in BPAEC via NO which is produced by increasing inducible nitric oxide synthase (iNOS) expression and activation of the signal pathway—NF-κ B. This study tried to investigate whether anisodamine could prevent BPAEC from TNF-α -mediated apoptosis and its mechanism related to modulating NF-κ B activity. Methods: BPAEC were treated with 2500U/ml TNF-α to induce apoptosis,. Anisodamine(0.2mg/ml) was added 30min prior to TNF-α -treatment. Electrophoretic mobility shift assay (EMSA) and western blots were performed on cell extracts to identify changes in NF-κ B activity , the presence of I-κ Bα (the NF-κ B-associated inhibitory protein), and the expression of iNOS. BPAEC apoptosis was determined by apoptotic morphological changes, DNA ladder pattern on agarose gel electrophoresis, and percentage of DNA fragmentation. Results: TNF-α treatment resulted in increased BPAEC NF-κ B activity (Fig.1), I-κ Bα degradation (Fig.2), iNOS expression (Fig.3) and apoptosis. Compared with control, Anisodamine decreased TNF-α -mediated NF-κ B activity (Fig.1), blocked I-κ Bα degradation (Fig.2), inhibited iNOS expression (Fig.3) and attenuated apoptosis. Conclusions: These data indicate that ① anisodamine has a protective effect on TNF-α -mediated BPAEC apoptosis; ② the inhibition of iNOS expression through NF-κ B/I-κ Bα pathway might contribute to the mechanism of cytoprotection of anisodamine.

Fig.1 EMSA showed NF-kB activity in BPAEC.

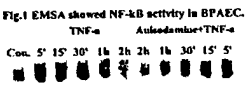


Fig.2 Western blot revealed the presence of I-kBα

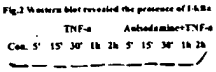
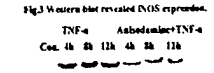


Fig.3 Western blot revealed iNOS expression.



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ACUTE SHOCK INDUCED LUNG INJURY IS INFLUENCED BY RESUSCITATION FLUIDS. M.M. Badellino*, R.F. Buckman, Jr.*, M.P. Shashikant* M.R. Wolfson* and S.I. Myers. Temple Univ., Phila., PA, 19140.

This study tested the hypothesis that resuscitation regimes would modulate end-organ pulmonary injury following hemorrhagic shock. Male Sprague Dawley rats (250-350gm) were hemorrhaged (MBP 40 mmHg) for 60 minutes (min) and resuscitated with either shed blood (SB), lactated ringer's (LR, 3 x SB volume) or hypertonic saline (HTS, 7.5% sodium chloride, 1/4 SB volume). A control group (Ctrl) was not bled or resuscitated. At 60 min, lungs were harvested, ventilated, and supported using an isolated perfused lung apparatus. Pulmonary vascular resistance (PVR) and the pulmonary capillary filtration coefficient (K_f) were calculated and values expressed as Mean ± SD (N=4, ^{a}-p<0.05 vs Ctrl; ^{b}-p<0.05 vs HTS and LR; ^{c}-p<0.05 vs HTS; ^{d}-p<0.05 vs LR, by KW ANOVA).

	PVR (mmHg/ml/min)	K_f (gm/min/mmHg/100gm)
Ctrl	0.20±0.26	0.0034±0.001
HTS	0.21±0.19	0.0031±0.001
LR	0.33±0.09	0.0050±0.002^{a,c}
SB	0.30±0.01	0.0299±0.010^{a,b,d}

Resuscitation results in functional, acute lung injury manifested as alterations in K_f. This injury is dependent on resuscitation regimes. Shed blood is associated with the greatest injury, whereas hypertonic saline attenuates acute lung injury. These data suggest that hypertonic saline may offer significant benefits in hemorrhagic shock resuscitation.

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MECHANISM OF HYPOXEMIA AFTER LPS ADMINISTRATION IN RABBITS. JE Baumgardner, IC Choi*, A Vonk-Noordegraaf*, HF Frasch*, GR Neufeld*, and BE Marshall*. University of Pennsylvania, Philadelphia, PA 19104

The multiple inert gas elimination technique (MIGET) has been used to determine the mechanisms of impaired gas exchange in ARDS in humans, in large animal models of ARDS, and in models of ARDS using isolated perfused lungs of small animals. V/Q distributions in intact small animal models of ARDS, however, have not been reported because the blood sample volume required for traditional MIGET by GC is approximately 20 ml. **Methods:** We developed micropore membrane inlet mass spectrometry (MMIMS) to measure inert gas partial pressures in small blood samples, and we used this new technique to investigate the mechanisms of impaired gas exchange after endotoxin administration in the intact rabbit. Arterial, PA, and peripheral venous catheters were placed in 11 New Zealand rabbits under pentobarbital anesthesia. Hemodynamics, arterial blood gases, and V/Q distributions from MIGET by MMIMS were measured at baseline and 1 hour after 5mcg/kg of LPS (Salmonella abortus equii). **Results:** Administration of endotoxin resulted in hypotension, pulmonary hypertension, reduced cardiac output, metabolic acidosis, and hypoxemia. V/Q distributions for normal rabbits were broad (logSD,Q=1.00±0.05) and skewed towards high V/Q regions compared to isolated perfused rabbit lungs. Endotoxin administration resulted in an increase in shunt from 7.9±2.0% at baseline to 14.2±1.7% (P=0.04), with no broadening of the V/Q distributions and no increase in perfusion to low V/Q regions. **Conclusions:** The mechanism of hypoxemia after endotoxin administration in this *in-vivo* model of ARDS in rabbits was an increase in shunt with little change in distributions of V/Q ratios.

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HELIUM/OXYGEN (H/O) COMPARED TO AIR/OXYGEN (A/O) IN A PORCINE MODEL OF OLEIC ACID- INDUCED ACUTE LUNG INJURY (ALI)

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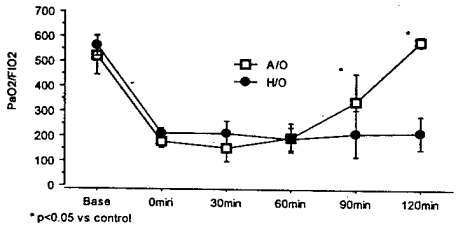
H/O mixtures have theoretical advantages compared to conventional A/O mixtures in the treatment of conditions marked by turbulent airflow. Our study was designed to determine the efficacy of H/O compared to A/O in improving oxygenation in an animal model of ALI.

Methods. Eight pigs were anaesthetized, intubated, ventilated, and oxygenated using an F_iO_2 of 0.40. After baseline measurements, oleic acid was infused intravenously (0.01ml/kg) until the PaO_2/F_iO_2 fell to <200 (0 min). In the A/O group (n=4), the F_iO_2 was then increased to 0.60. While maintaining an F_iO_2 of 0.60, 5, 10 and 15 mmHg of PEEP was added at 30 minute intervals. In the H/O group (n=4), the heliox mixture (60% helium-40% oxygen) was started at

0 min and data collected at the same time points. PEEP was not added to the Heliox group.
Results. Results are reported as mean±SD.



Conclusion: H/O had no significant advantage over conventional A/O mixtures with PEEP on the PaO_2/F_IO_2 ratio in this animal model of ALI. Turbulent gas flow in injured airways may not be a significant contributing factor in ALI hypoxemia.

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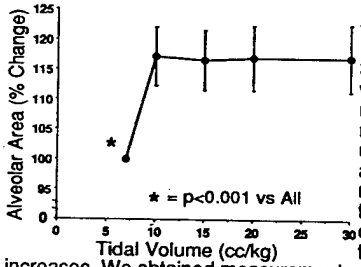
ALVEOLAR OVER-DISTENTION DOES NOT OCCUR DESPITE SUPRA-PHYSIOLOGIC TIDAL VOLUMES Ulysse G. McCann II,* Henry J. Schiller,* David E. Carney,* Louis A. Gatto,* Andrew M. Paskanik,* Gary F. Nieman. Department of Surgery, State University of New York Health Science Center at Syracuse, NY 13210.

Introduction: Alveolar over-distention due to volutrauma has been proposed as the mechanism of ventilator induced lung injury (VILI). However, in previous experiments with pulmonary *in vivo* microscopy we have never observed alveolar over-distention. Therefore, we hypothesized that alveolar over-distention does not occur even at supra-physiologic tidal volumes. **Methods:** Yorkshire pigs (n=4) were anesthetized for a right thoracotomy. Sub-pleural alveoli (n=26/tidal volume) were directly assessed by *in vivo* microscopy (100x) as animals were ventilated over a range of tidal volumes (7, 10, 15, 20, and 30cc/kg body weight). The microscopic field remained unchanged allowing measurement of the same alveoli over consecutive tidal volume increases. We obtained measurements at end-inspiration by computer-video image analysis. Data are presented as mean ± SEM, unpaired t-test. **Results:** Alveolar areas increased significantly from 7cc/kg to 10cc/kg with no changes thereafter (Figure). **Conclusion:** Sub-pleural alveoli increase in size over the physiologic range of 7cc/kg to 10cc/kg tidal volume, while supra-physiologic tidal volumes do not cause alveolar over-distention even up to 30cc/kg. These data suggest that alveolar over-distention is not a mechanism of VILI.



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PULMONARY CLEARANCE OF ADRENOMEDULLIN IS REDUCED DURING THE LATE STAGE OF SEPSIS. D. Orman*, I. H. Chaudry, and P. Wang, Brown University School of Medicine and Rhode Island Hospital, Providence, RI 02903.

Sepsis is characterized by an early, hyperdynamic phase followed by a late, hypodynamic stage. Although upregulation of adrenomedullin (ADM), a novel potent vasodilatory peptide, plays a major role in producing hemodynamic responses during sepsis, it remains unknown whether the tissue clearance of this peptide is altered under such conditions. To determine this, male adult rats were subjected to sepsis by cecal ligation and puncture (CLP) followed by fluid resuscitation. At 5 h (i.e., the hyperdynamic phase of sepsis) or 20 h (i.e., the hypodynamic phase) after CLP, the animals were injected with ^{125}I-labeled ADM (~300,000 cpm/rat) via the jugular vein. A blood sample was drawn 30 min after the injection and the rats were perfused with normal saline to remove blood. Organs such as the lungs, kidneys, gastrointestinal tract, pancreas, spleen, liver, brain, skeletal muscle, and skin were harvested and their radioactivity (i.e., cpm) was measured. The tissue distribution of ^{125}I-ADM was then calculated as the percentage of the total cpm per gram of tissue (%/g). Some results (mean ± SE) at 20 h after CLP are:

	Lung	Blood	Heart	Spleen	Gut
Sham	20.46±.75	0.51±.02	0.19±.02	0.22±.01	0.16±.01
CLP	11.99±2.0*	0.65±.02*	0.33±.03*	0.38±.04*	0.24±.01*

(n=7/group, unpaired Student's t-test: *P<0.05 vs. Sham)

The results indicate that there were no significant changes in ^{125}I-ADM distribution at 5 h after CLP, compared to shams. At 20 h after CLP, however, there was a marked decrease in radioactivity in the lungs. In contrast, significantly increased radioactivity was observed in all other organs except the liver and kidneys. The pulmonary distribution of ^{125}I-ADM was found to be far greater than in any other tissue tested. In separate groups of animals, injection of the ^{125}I-ADM into the left ventricle at 20 h after CLP resulted in a significant decrease in radioactivity in the lungs of both sham and septic animals. These results suggest that the lungs are the primary site of ADM clearance, which is diminished in late sepsis. The decreased pulmonary clearance of ADM may play an important role in maintaining the elevated levels of plasma ADM under such conditions (Supported by NIH GM 57468).

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INHIBITION OF POLY(ADP-RIBOSE) SYNTHETASE (PARS) ATTENUATES LPS-INDUCED PULMONARY VASOMOTOR DYSFUNCTION EJ Pulido*, BD Shames*, HA Barton*, DD Bensard*, RC McIntyre Jr. Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Oxidant injury leads to NAD^+ and ATP depletion by excessive activation of the nuclear enzyme PARS. This energetic failure has been proposed as a major pathway in the vascular dysfunction in endotoxemia/sepsis. Endotoxin (LPS) causes an acute lung injury characterized by dysfunction of pulmonary vasorelaxation, neutrophil (PMN) accumulation, edema, and ATP depletion. We **hypothesized** PARS inhibition attenuates LPS-induced acute lung injury. The **purpose** of this study was to determine the effect of PARS inhibition after LPS on: 1) endothelium-dependent (ED; response to acetylcholine) and endothelium-independent (EI; response to sodium nitroprusside) vasorelaxation, 2) lung PMN accumulation, 3) lung edema, and 4) lung ATP levels. **Methods:** Rats were injected i.p. with saline or LPS (20 mg/kg) and then with the PARS inhibitor 3-aminobenzamide (3-AB, 10 mg/kg) 90 min later. After 6 h, vasomotor function was assessed in isolated pulmonary artery (PA) rings (expressed as percent maximum relaxation of the preconstricted tone), PMN accumulation by myeloperoxidase activity, edema by wet/dry weight ratios, ATP by enzymatic assay (nmol/mg protein), and PARS activity in the PA by conversion of labeled NAD to ADP (nmol/min/µl). Data analyzed by ANOVA. **Results:** PARS inhibition attenuated LPS-induced impairment in ED and EI vasorelaxation and preserved lung ATP. PARS inhibition didn't affect LPS-induced increases in PMNs and edema (not shown). PARS activity increased five-fold after LPS; *in vivo* 3-AB abrogated this increase.

Group	*ED*	*EI*	*ATP*	*PARS activity*
Control	94%	99%	12.8 ± 0.3	0.22 ± 0.02
LPS	41%*	58%*	8.7 ± 0.4*	1.22 ± 0.12*
LPS+3-AB	63%*‡	79%*‡	12.5 ± 0.5	0.25 ± 0.02

n=8 rings/4 rats per group, *P<0.05 vs. Control, ‡ vs. ETX

Conclusion: These data suggest that depletion of cellular energy stores by PARS activation represents an important mechanism in LPS-induced pulmonary vasomotor dysfunction. However, PARS inhibition had no effect on LPS-induced lung PMN accumulation and edema.

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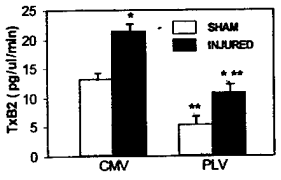
EFFECT OF VENTILATORY STRATEGY ON PULMONARY THROMBOXANE RELEASE FOLLOWING INTESTINAL ISCHEMIA-REPERFUSION(I/R) M. P. Shashikant*, B.Cooper*, A.Riva*, R.Milner*, M.M.Badellino*, L. Bartula*, S.I.Myers, T.H.Shaffer*, M.R.Wolfson*, Temple Univ Sch Med, Phila., PA 19140

I I/R induced lung injury has been associated with upregulation of endogeneous pulmonary thromboxane A_2 (TxA_2) release, a contributor to increased microvascular dysfunction and pulmonary vascular resistance (J Appl Physiol82(2):592-8,1997).In addition,conventional mechanical ventilation (CMV)as compared to spontaneous breathing has been shown to decrease microvascular permeability associated with this insult (Shock, 9:62,1998) and partial liquid ventilation (PLV) with perfluorochemical liquids shown to decrease lung injury associated with ARDS as compared to CMV(J Appl Physiol 84(2): 624-40,1998). In this study, we evaluated the effect of positive pressure ventilatory strategies on pulmonary TxA_2 release. Anesthetized rats were supported by CMV or PLV and underwent 60 min of sham or superior mesenteric artery occlusion and 60 min of reperfusion.The lungs and heart were removed *en bloc*, continually ventilated, and supported with a non-recirculating isolated perfused lung apparatus.TxB_2, stable metabolite of TxA_2, in the pulmonary effluent was analyzed by EIA,and normalized to volume and perfusate rate.Data were analyzed with ANOVA as a function of injury and ventilatory strategy. (* vs injury $p < 0.05$, ** vs ventilatory strategy $p < 0.01$).The results demonstrate that 1) TxB_2 increased following I I/R injury; 2) TxB_2 was lower during PLV as compared to CMV, independent of injury. These data suggest that PLV may provide lung protection during I I/R injury associated with decreased release of a potent pulmonary vasoconstrictor.



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DECREASED TRANSCRIPTION OF SURFACTANT PROTEINS IN AN ANIMAL MODEL OF THE ADULT RESPIRATORY DISTRESS SYNDROME (ARDS)
Gregory Schears, Jian Zheng,* Clifford Deutschman., Department of Anesthesia, University of Pennsylvania, 19104

ARDS is a poorly understood, prevalent condition with a high mortality. Over the past 30 years therapeutic strategies have had little impact. Surfactant activity is deficiant in ARDS. Bronchoalveolar lavage specimens from humans with ARDS have shown a reduction in surfactant proteins A (SP-A) and B (SP-B), essential for normal surfactant function. We hypothesize that a decrease in transcription was responsible for this decrease in surfactant proteins activity. Cecal ligation and double puncture (CLP) or sham operation (SO) were performed in male Sprague-Dawley rats. Lung tissue obtained at 0, 3, 6, 16, 24, 48, and 72 hours was prepared for histological analysis, Northern Blot Hybridization, and Transcription Elongation Analysis. Data were collected on 3 rats per time point. Signal density at each point was averaged and ANOVA with Tukey's post hoc test was performed with $p<.05$ indicating significance. H & E stained CLP lung tissue showed septal thickening, neutrophilic infiltration and hyaline membrane deposition over time. At 24hrs post CLP, transcription of SP-A, SP-B, and SP-C decreased 2 fold relative to SO, and steady state SP-A mRNA levels are decreased by 20% relative to SO. We demonstrated a significant, sepsis associated reduction in transcription of SP-A, SP-B, and SP-C and a decrease in RNA for SP-A in CLP compared to SO. This suggests a possible mechanism for reduced surfactant activity seen in lavage fluid of humans with ARDS.

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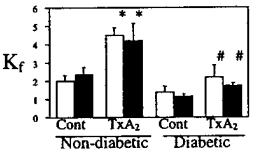
STREPTOZOTOCIN (STZ)-INDUCED DIABETES BLUNTS THE PULMONARY MICROVASCULAR RESPONSE TO THROMBOXANE A_2(TxA_2). JK Wright*, JC Falck*, D Murray*, LT Kim*, F. Nwariaku*, & RH Turnage. Univ. of Texas Southwestern Medical Center, Dallas, TX 75216.

The purpose of this study was to determine the effect of STZ-induced diabetes mellitus on the pulmonary microvascular response to TxA_2 and to examine the effect of perfusate osmolarity and glucose concentration on TxA_2-mediated changes in microvascular permeability. Sprague-Dawley rats received STZ (65 mg/kg, i.p.) or vehicle(NS, 0.2 ml, i.p.). Four weeks later the lungs of these animals were perfused *ex vivo* with Krebs-Henseleit buffer containing 3% albumin and either 120 or 600 mg/dl glucose. Mannitol (480 mg/dl) was used in the normoglycemic group as an osmotic control (340 mOsm in both groups). The lungs were perfused for 15 min. with or without the TxA_2 receptor agonist U-46619 ($1.4x10^{-7}$ M) after which vascular permeability was determined by measuring the capillary filtration coefficient (K_f) using a gravimetric technique. The data are expressed as mean± SEM & analyzed with AVOVA.



**p<0.01 vs. non-diabetic control*
#p<0.02 vs. non-diabetic with TxA_2
K_f=g/mmHg/min/100g lung
n = 3 – 5 per group

These data demonstrate that the lungs of diabetic animals respond differently to TxA_2 receptor activation than do those of normal animals. Furthermore, this difference is not altered by *ex vivo* perfusion with hyperglycemic or hyperosmolar buffers. These data provide further evidence that the altered pulmonary response to pro-inflammatory mediators in diabetics is due to inherent properties of the lung.

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MIP-2 chemokine mRNA and protein expression is altered by G-CSF prophylaxis in septic rats
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Recombinant human granulocyte colony-stimulating factor (rhG-CSF, Filgrastim) increases in rats with abdominal contamination and infection (ACI) survival rate and alters also granulocyte functions and cytokine expression. Therefore MIP-2 (macrophage inflammatory protein-2) a chemotactic factor for granulocytes was studied in tissue specific mRNA expression and at the protein level in the plasma and in the peritoneum after G-CSF prophylaxis. A sepsis model (1), modelling clinical complexity (2) with anaesthesia, antibiotic prophylaxis (10 mg/kg co-amoxiclav), volume loading, laparotomy and ACI with a standardized human stool inoculum, analgesia and G-CSF prophylaxis (20 µg/kg, 12 h before and after ACI) was used. Total RNA was prepared by the method of Chomcziski from tissue samples obtained 24 h

after ACI. MIP-2 (4 rats/group) was quantified in competitive RT-PCR using a rat cytokine competitor plasmid, agarose gel electrophoresis, ethidium bromide staining and digital imaging. MIP-2 protein levels were determined by ELISA (Biosource).

Study groups	MIP-2 mRNA [pg]			MIP-2 protein [pg/ml]	
(n = 4/group)	lung	liver	spleen	plasma	peritoneal
Control (no ACI)	0.9	0.87	0.83	48.5	n.d.
ACI	0.2	0.35	0.45	89.2	n.d.
ACI + AB	0.3	0.06	0.15	117.4	2193
ACI+AB+G-CSF	0.6	1.07	1.73	66.5	6211

MIP-2 mRNA expression was reduced by ACI. An increase of MIP-2 obtained by G-CSF was not seen after antibiotic prophylaxis alone. Systemic MIP-2 protein was reduced and increased locally in the peritoneum. Beneficial effects of G-CSF, such as recruitment of granulocytes toward the side of infection and reduction of unspecific intravascular inflammation (2), can be largely explained by an altered distribution of MIP-2. 1) Lorenz et al Surgery 1994, 116; 925 2) Bauhofer et al 1998 in Cytokines and the abdominal surgeon, eds. Schein et al, Landes, Austin pp117-141.

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ENHANCED EXPRESSION OF IL-18 IN SEPTIC PATIENTS – QUANTIFICATION OF mRNA-LEVELS

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I. Department of Surgery, University of Cologne, 50931 Cologne, Germany

Introduction: The role of proinflammatory Interleukin-18 (IL-18) in the pathogenesis of human sepsis has yet to be determined. IL-18 augments IFN-γ production by spleen cells and enhances natural killer cell cytotoxicity. We quantified IL-18-mRNA levels of human lymphocytes in septic ICU patients using healthy volunteers as controls.

Methods: Blood samples were taken from ICU patients fulfilling at least 3 SIRS criteria (n=5), and from healthy volunteers (n=5). Total RNA was extracted from peripheral blood lymphocytes (PBL) obtained by Ficoll-Hypaque density gradient separation. RT-PCR was performed on 1 µg of total RNA amplifying a 330-bp product. Quantification was achieved using HPLC with GAPDH (487 bp) as internal standard.

Results: Compared to healthy volunteers there was a fourfold increase of IL-18-mRNA levels in septic ICU-patients. More severe cases expressed higher levels of IL-18-mRNA. Significantly lower IL-18-mRNA levels were detected in PBL of healthy volunteers.

Conclusion: IL-18-mRNA expression is elevated in PBL of septic ICU patients. Quantification of IL-18 serum concentration by ELISA and time course experiments should provide more information about this proinflammatory cytokine and its role in the pathogenesis of human sepsis.

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ALCOHOL EFFECTS ON INTESTINAL BARRIER FUNCTION AND INFLAMMATORY CYTOKINE PRODUCTION.

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The gut may be an important reservoir for infection and the initiation of SIRS following shock/trauma. Ethanol (EtOH) intake increases the incidence of septic complications following trauma. The effects of EtOH exposure on intestinal barrier function and cytokine production are unknown and were studied *in vitro*.

Methods: CaCO2 cell monolayers grown to confluence in a two chamber culture system were challenged with 10^8 CFU/ml *Escherichia coli* C25 with or without EtOH (0%, 1%, 3%). Basal media was cultured at 60 and 240 minutes to detect bacterial translocation (BT, $\log_{10}$ CFU/ml). Apical media were assayed at 240 minutes for IL_6 and TNF∝ (pg/ml ± SD by ELISA). Monolayer integrity was confirmed by serial measurement of transepithelial electrical resistance. Permeability was studied using 10KD and 40KD dextran probes.

Results:

EtOH	0%	1%	3%
Bacterial Translocation ($\log_{10}$ CFU/ml, n=8)			
	2.92 ± 0.04	3.21 ± 0.03	3.37 ± 0.05*
Cytokine Data (n = 10-15 each)			
IL_6	63.9 ± 32.3	97.3 ± 14.9*	131.5 ± 29.2*
TNF∝	50.7 ± 34.8	0.43 ± 1.5*	32.4 ± 10.9

* $p < 0.05$ vs. no EtOH # $p < 0.01$ vs. 1% or 3% EtOH

* $p < 0.05$ vs. no EtOH; * $p < 0.001$; # $p < 0.001$ vs. 1% or 3% EtOH

No increase in permeability to either probe was noted up to 240 minutes in culture.

Conclusions: EtOH had disparate effects on TNF and IL_6 release from CaCO2 cells following bacterial challenge. These effects may be associated with impaired barrier function against BT.

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DIFFERENTIAL PHENOTYPIC EXPRESSION OF PRO- AND ANTI-INFLAMMATORY MEDIATORS ARE INDUCED BY ENDOTOXIN TOLERANCE *IN VIVO* AND *IN VITRO*.

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Pre-exposure to endotoxin (LPS) *in vivo* or *in vitro*, diminishes responsiveness to subsequent exposure to LPS. This phenomenon known as LPS tolerance involves alterations in the production of inflammatory mediators. Efforts to elucidate mechanisms responsible for tolerance have focused on the balance of production of pro- and anti-inflammatory mediators. We hypothesized that tolerance induction *in vivo* or *in vitro* produces a common cellular phenotype of augmented anti-inflammatory and suppressed pro-inflammatory mediator production. For tolerance induction *in vivo*, rats were given daily consecutive intraperitoneal injections of LPS (0.05mg/100g body weight and 0.25mg/100g). After two days, peritoneal macrophages (MØ) were harvested by lavage. For tolerance induction *in vitro*, NR8383 cells (rat alveolar MØ) were desensitized by exposure to LPS (1 µg/mL) for 24 hours. Cells were then washed and stimulated for 24 hours with 10µg/mL LPS. *In vivo* and *in vitro* supernatants were extracted for analysis of the pro-inflammatory mediators thromboxane (TxB_2) or tumor necrosis factor alpha (TNFα), the anti-inflammatory cytokine interleukin-10 (IL-10), and the mediator nitric oxide (NO). LPS stimulated rat peritoneal MØ production of TxB_2 was decreased 93% from 33.3 ± 2.4 ng/mL to 4.3 ± 0.7 ng/mL ($p<0.01$, n=3). By contrast, IL-10 production was increased 1.5 fold from 61.5 ± 16.5 pg/mL to 171.2 ± 9.3 pg/mL ($p<0.01$; n=3). NO synthesis, measured by nitrite, was attenuated from 4,600 ± 792.3 ng/mL to 2,330 ± 224.8 ng/mL ($p<0.01$, n=4). *In vitro* LPS tolerance induction in NR8383 cells produced a phenotypically similar pattern. TNFα production decreased 99% from 50.7 ± 3.9 ng/mL to 0.78± 0.13 ng/mL in desensitized alveolar MØ compared to control cells ($p<0.01$, n=4). Basal IL-10 production was augmented 5 fold in LPS tolerance relative to control basal ($p<0.01$, n=4), and basal NO production was increased 3 fold in tolerance ($p<0.01$, n=4). The data support the hypothesis that *in vivo* or *in vitro* tolerance produces a common phenotype of suppressed pro-inflammatory mediator and augmented IL-10 production by MØ. The latter may produce autocrine suppression of pro-inflammatory mediator production in tolerance.

Supported by NIHGM27673.

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MIXED VENOUS AND BRONCHOALVEOLAR LAVAGE (BAL) FLUID IL-6 PREDICTS SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) IN PATIENTS UNDERGOING PHOTODYNAMIC THERAPY (PDT).

Beth Gehan*, Arthur R. Bouwman*, Yoram G. Weiss*, Clifford S. Deutschman, Department of Anesthesia, University of Pennsylvania, Philadelphia, PA 19104

Debulking surgery combined with PDT is an experimental treatment for patients with disseminated intraperitoneal tumors. A predictably high percentage of these patients develop SIRS, often accompanied by acute lung injury (ALI). PDT provides a uniform model for the study of the processes accompanying the development of SIRS and ALI in humans. Cytokines may be important in the development of SIRS/ALI. Therefore, we hypothesize that, at a certain critical point in time, we will be able to recognize a distinct difference in the serum and BAL inflammatory mediator profiles when comparing patients that develop SIRS/ALI to those who do not. After IRB approval, blood and BAL fluid samples from nine patients undergoing PDT were analyzed. IL-6 levels were measured by enzyme-linked immunosorbent assay kits (ELISA). All measurements were performed in duplicate. IL-6 levels increased significantly in both groups immediately after surgery and PDT. In patients who went on to recover uneventfully, levels returned to normal by 16 hours post-op. However, in patients that developed SIRS, IL-6 levels in both blood and BAL fluid continue to rise 16 hours post-op. We conclude that progressive elevations in serum and BAL IL-6 may be a marker for patients at risk to develop SIRS and ALI.

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CYTOKINE LEVELS IN MENINGOCOCCCAL SEPSIS: NOT ALL ARE RELATED WITH SURVIVAL. J.A. Hazelzet, R.F. Kornelisse * , K.F.M. Joosten * , R de Groot * , C.E. Hack * . Sophia Children's Hospital, Rotterdam; Central Laboratory Blood Transfusion Services, Amsterdam, The Netherlands.

Meningococcal septic shock (MSS) is a serious life-threatening disease in otherwise healthy children and young adults. MSS was one of the first diseases in which circulatory TNF was detected, later other cytokines were also described. The levels of those cytokines were related to survival. Remarkable is the rapidly progressive character of the disease. Normally mortality occurs within the first 72 h. We were interested in the time course of the cytokine levels, and their relation with the development of clinical symptoms. All patients admitted to the PICU with a MSS were included, when the development of petechiae was not longer than 12 h before admission. Arterial blood was sampled at admission, 24 and 72 h later. TNF, IL-6, 8, 10, and 12 were determined. In the period of 1991 - 95, 54 patients were admitted, age 3.3 y (3m-17.9y); 15 patients died (non-S). Only 1 patient died after 72 h. The median duration of petechiae was lower in non-S (4.7h vs 6.9 h, p=0.02). On admission, all cytokine levels were significantly higher in the non-S; however, when these levels were corrected for the duration of petechiae, only IL-6, 8, and 12 remained significantly higher. At 24 h only the levels of IL-6 were different between S and non-S. In conclusion, when analyzing cytokine levels, the duration of petechiae before admission needs to be taken into consideration.

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ROLE OF PLA_2 AND PKC ISOFORMS IN THE CARDIAC EFFECTS OF TNF-α AND IL-1β. R.H. Kennedy*, J. McHowat* and S.J. Liu*, (Spon: J.A. Majde). Univ. Arkansas for Med. Sciences, Little Rock, AR 72205

Interleukin (IL)-1β and tumor necrosis factor (TNF)-α have been shown to decrease L-type calcium channel current (I_{Ca}) in rat ventricular myocytes. In addition, we have demonstrated that IL-1β enhances membrane-associated Ca-dependent phospholipase A_2 (PLA_2) activity in these cells. Current studies were designed to further elucidate the signaling pathways underlying the cardiac actions of these cytokines. Experiments in cells pre-exposed to 5 ng/ml IL-1β showed that 10 ng/ml TNF-α can elicit further increases in both cytosolic and membrane-associated PLA_2 activities, as well as in arachidonic acid (AA) release. This suggests that TNF-α and IL-1β act via different PLA_2 isoforms, and the presence of these isoforms was verified by Western blot analysis. The possible role of AA in the cardiac effects of the cytokines was supported by whole-cell patch-clamp studies which showed that AA, like TNF-α and IL-1β, decreases I_{Ca} in a dose-dependent manner. Other experiments examined the possible role of PKC isoforms in the cardiac actions of the two cytokines. Myocytes were incubated for 5 min with 5 ng/ml IL-1β, 10 μM phenylephrine (PE) or 100 nM phorbol 12-myristate 13-acetate (PMA). Western blots showed that IL-1β and PE, but not PMA, caused translocation of PKC-ζ from the cytosolic to the particulate fraction. Phorbol 12,13-dibutyrate (PDBu; 100 nM) decreased I_{Ca} to an extent similar to that elicited by IL-1β. However, IL-1β decreased while PDBu increased the inactivation of I_{Ca}. These data indicate that both PLA_2 and PKC are involved in the signaling pathways underlying the cardiodepressant actions of the cytokines; however, further work is required to delineate the exact mechanisms coupled to each cytokine as well as possible crosstalk among the pathways.

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IgG-COATED ERYTHROCYTES AUGMENT THE LPS-STIMULATED INCREASE IN SERUM TNF LEVELS VIA Fcγ RECEPTORS. D.J. Loegering and M.L. Refici Department of Physiology & Cell Biology, Albany Medical College, Albany, NY 12208.

Phagocytosis of erythrocytes coated with IgG (EIgG) have been used as a model of thermal injury-induced erythrocyte phagocytosis. Injection of EIgG has been shown to increase the mortality due to LPS and to cause a 10-fold augmentation of the LPS-stimulated increase in serum TNF levels. The present study found that EIgG prime mice for the serum TNF response to LPS to the same extent as that previously seen in rats. The prior injection of EIgG also augmented (10-fold) the increase in serum TNF levels caused by peritonitis (cecal ligation and puncture). Heat damaged erythrocytes, which may be cleared by an FcγR-mediated mechanism, caused nearly the same degree of priming as EIgG. The role of FcγR in vivo was determined using gamma chain deficient mice that lack functional FcγRI and FcγRIII. In controls, the prior injection of EIgG caused a 13-fold augmentation of the LPS-stimulated increase in serum TNF levels while the deficient animals showed only a 2-fold increase.

Hepatic uptake of the EIgG was reduced 80% in the FcγR deficient animals. These results indicate that FcγR signaling can prime macrophages for an exaggerated cytokine response to LPS or bacterial infection. (Supported by NIH-GM-50368)

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HYPOTHERMIC EFFECTS ON MEDIATOR RELEASE IN RAT MICROGLIA STIMULATED BY ENDOTOXIN (LPS).

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Therapeutic moderate hypothermia has neuronal protection against brain injury. Microglias, an immune-related cells in the brain, may be involved in subsequent neuronal damages after the injury. We examined effects of temperature(37, 33 and 30°C) on mediator release (NO, IL-6 and TNF-alpha) from activated microglias.

Cultured microglia from neonatal rats were used for evaluating the following indices: production of nitric oxide mesured by the methods of acetyl-cytochrome c reduction and nitrite accumulation in the culture medium, respectively, and IL-6, TNF-alpha by ELISA.

At 30 and 33°C, nitric oxide production stimulated by LPS was decreased as low as 10, 30% of control at 37°C and IL-6 production was also decreased to 30, 40% of the control. Besides TNF-alpha release at 30°C was depressed from three hours after the stimulation but such depression in the other mediators occurred later. However subsequent changes of TNF-alpha production at 30°C were not different from those at 37 °C.

The present study indicates that 1) hypothermia inhibits nitric oxide and IL-6, productions of activated microglias, and that 2) the degrees or time course changes in the mediator depressions were different. Therefore, further studies are required to define such different changes in the mediators in the mechanisms of the neuroprotective effects of cerebral hypothermia against brain injury.

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PLASMA LEVELS OF CYTOKINES AND CHEMOKINES ARE UNALTERED IN SEPTIC ICU PATIENTS: RE-APPRAISAL OF THEIR IMPORTANCE

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Objective: To measure the levels of TNFα, IL-1β, IL-10, MIP-1α, MCP-1 and Gro-α in sera of ICU patients with sepsis as defined by the American College of Chest Physicians/Society of Critical Care Medicine.

Design: A prospective case series study.

Patients: 20 septic patients on surgical ICU and 10 healthy volunteers.

Methods: At the onset of 3 SIRS criteria, blood samples were drawn from all patients and mediator levels were measured for a period of 3 days using highly specific human ELISA kits.

Results: Apart from a modest 3 fold increase in MIP-1α-levels in septic patients as compared to healthy volunteers (15 pg/ml vs. 5 pg/ml respectively, $p < 0.01$), cytokine and chemokine levels were not significantly elevated in septic ICU patients.

Conclusion: This data seriously questions the role and significance of cytokines or chemokines in the pathophysiology of sepsis. It might help to explain the lack of clinical efficacy of anti-cytokine strategies in sepsis. Consequently, alternative approaches need to be considered to counteract the consequences of sepsis and septic shock.

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ADENOVIRAL-MEDIATED OVEREXPRESSION OF IL-10 IMPROVES SURVIVAL OF SEPSIS.

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Background and Objective: IL-10 is reported to be a cytoprotective cytokine. Here we investigated whether overexpression of IL-10 provided by adenoviral gene transfer could prevent survival of panperitonitis in rats. Materials & Methods: Adenovirus (Adex/IL-10) which transfers IL-10 gene and produce IL-10 was made. Study 1: One hour after cecum ligation and puncture(CLP), Adex/LacZ(n=12) or Adex/IL-10 (n=12) was injected into the spleen of male Wistar rats. White blood cell count, CRP, plasma IL-6 and TNFα levels at 24 and 48 hrs. after CLP and the survival rate were examined. Study 2: Three hour after CLP, Adex/LacZ(n=12) or Adex/IL-10 (n=12) was injected. The plasma IL-6 and TNFα levels at 24 and 72 hrs after CLP and the survival rate was examined. Results: Study 1 (Table 1): Although induction of IL-10 did not change these parameters significantly, it significantly improved survival of rats comparing to the control (median survival time: 28 days in IL-10 vs 6 in control, p=0.008). Study 2 (Table 2): No significant difference existed in cytokine productions and survival between Adex/IL-10 and Adex/LacZ (median survival time: 5 days in IL-10 vs 4 in control, p=0.42). Conclusions: Overexpression of IL-10 by adenoviral gene transfer at early stage of panperitonitis may improve outcome of sepsis.

Table 1. Parameters at 48hrs after CLP in Study 1 (mean±SE)

	WBC(/μl)	CRP(mg/dl)	IL-6(pg/ml)	TNFα(pg/ml)
Adex/LacZ	5291±689	323±221	67±26	33±4
Adex/IL-10	8067±1261	533±256	29±11	27±2

Table 2. Serum IL-6 and TNFα at 24 and 72hrs after CLP in Study 2 (mean±SE) (pg/ml)

	IL-6 (24h)	IL-6 (72h)	TNFα(24h)	TNFα (72h)
Adex/LacZ	69±44	1034±864	5±2	59±50
Adex/IL-10	13±12	104±95	3±1	6±3

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IMMUNOPATHOLOGY OF A 2 HIT MODEL OF PULMONARY INJURY.

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A 2 hit mouse model was developed to investigate acid aspiration lung injury following bacterial peritonitis. Mice were subjected to non-lethal, cecal ligation and puncture (CLP). After 48 hours, the mice received an intratracheal (IT) injection of an acidic solution and were sacrificed at 3, 5, 8, 15, and 24 hours after acid. Bronchoalveolar lavage (BAL) fluid PMN counts and albumin concentrations, indicative of

lung injury reached maximum levels at 8 hours. Concentrations of the murine chemokine KC were highest at 3 hours in lung (0.5±0.2 ng/ml) and plasma (13.7±7.5 ng/ml) and then declined. Likewise, plasma MIP concentration was highest at 3 hours (2.4±.9 ng/ml) and declined by five hours. BAL MIP levels were highest at five hours (0.1±.05 ng/ml) with no significant differences between time points. To examine the potential synergistic effect of the two hits at 8 hours, additional animals were randomized into four groups: Saline, Acid, CLP-Saline, and CLP-Acid. BAL PMN counts were significantly greater in both acid groups and highest in the CLP-Acid group. Albumin levels were higher in the acid groups compared to the saline groups. MIP concentrations in plasma and lung were significantly elevated in the CLP-Acid group compared to other groups while KC levels were not. Finally, to investigate the role of MIP and KC, mice were pre-treated with either anti-KC, anti-MIP, or control antiserum. There were no significant differences in albumin concentration or plasma/lung KC between the groups. The mean BAL PMN count was lower, although not statistically significant, in animals treated with anti-MIP antiserum. However, BAL PMN counts were significantly decreased in the anti-KC treatment group. In the CLP-Acid model of acid aspiration lung injury, the effects of the two hits appear to be additive, not synergistic. Recruitment of PMNs into the lung was preceded by high local levels of KC and neutralized by anti-KC antibodies, suggesting KC has a major role in acid aspiration lung injury.

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ENDOTOXIN STIMULATES THE CYTOKINE-INDUCED PRODUCTION OF PROSTAGLANDIN E2, NITRIC OXIDE AND EXPRESSION OF EP1 RECEPTOR SUBTYPE IN AMNION AND CHORION EXPLANTS.
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Introduction: One of the predisposing factors that may lead to sepsis in pregnancy is chorioamnionitis. Endotoxin (LPS) is known to cause increased production of cytokines, nitric oxide, and prostaglandins in human amniochorionic membrane and elevated LPS levels are found in amniotic fluid of patients with infection-induced preterm labor. The purpose of this experiment was to localize the sites of these effects and study the effect of LPS on the membrane prostaglandin E receptor subtype EP1 in chorion and amnion explants. **Methods:** Human amnion and chorion whole tissue explants were incubated in culture media with increasing concentrations of LPS (0, .1, 1, 2 µg/mL) for 2 hours. Following incubation, the culture fluid was removed and assayed for interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), NO, and PGE2. Tissues were evaluated for changes in EP1 expression by Western blot analysis. **Results:** Incubation of amnion explants with LPS resulted in increased levels of NO, PGE2, TNF-α, and the expression of EP1 receptor protein, with little effect on IL-1β production. Incubation of chorion explants with LPS resulted in substantial dose-response increases in TNF-α, IL-1β ,and the EP1 receptor protein. No changes in NO levels or PGE2 were observed. A significant correlation was observed between PGE2 and NO levels in amnion (r=0.90) but was not present in chorion. **Conclusions:** Exposure of amniochorionic membranes to LPS results in a cytokine-induced increase in NO and PGE2 in amnion. LPS also causes the cytokine-induced expression of the EP1 receptor in both amnion and chorion. The effect of infection involving LPS during pregnancy may be due to a pleiotropic ability of LPS to induce both NO-induced PGE2 production and PGE EP1 receptor levels.

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ORALLY ADMINISTERED IL-6 PROTECTS THE GUT FROM HEMORRHAGE-INDUCED APOPTOSIS.
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Orally administered IL-6 restores intestinal barrier function following hemorrhage in both rat and mouse models. TUNEL and p53 immuno-histochemical staining, in situ hybridization for *bcl-2* mRNA localization, and RPA analyses of *fas* , *bcl-2* and caspases 1 and 2 mRNA expression were used to examine intestines from mice hemorrhaged and fed saline or IL-6 and enterocytes (IEC-6) exposed to hypoxia and LPS alone or LPS and IL-6 in vitro. Intestinal sections from mice hemorrhaged and fed IL-6 showed reduction in apoptosis and increases in *bcl-2* gene expression. IEC-6 cells exposed to hypoxia and LPS had high numbers of TUNEL positive cells, increased *fas* expression and decreased *bcl-2* expression. Subsequent exposure to IL-6 after hypoxia and LPS reduced apoptotic cell numbers, increased *bcl-2* expression and decreased *fas* expression. The data show that exposure of intestinal epithelial cells to IL-6 either by oral administration in hemorrhaged mice or co-culture following hypoxia and LPS treatment results in increased *bcl-2* gene expression, decreased *fas* expression and reduced damage from apoptosis.

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MANIPULATION OF ENDOGENOUS ADENOSINE MODULATES SERUM TUMOR NECROSIS FACTOR-ALPHA (TNF-α) DURING SEPSIS IN RATS
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Endogenous adenosine (ADO) is known to modulate macrophage TNF-α production *in vitro*. We previously reported that during sepsis, endogenous ADO plays a significant role in determining resting vascular resistance in selected regions *in vivo*. In the current study, we tested the hypothesis that manipulation of endogenous ADO during sepsis would modulate serum TNF-α concentrations *in vivo*. Male, SD rats (350-400 g) were made septic by IP introduction of a 200 mg/kg cecal slurry. At the time of sepsis induction, rats were treated with the ADO deaminase inhibitor pentostatin (PNT; n=5), the ADO receptor antagonist 8-sulfophenyltheophylline (SPT; n=5), or vehicle (VEH; 0.9% NaCl; n=6). Serum TNF-α (pg/ml) was determined at 4 and 24 hours after sepsis induction by ELISA. Significant differences from the VEH treated group over time ($p \leq 0.05$) were determined by 2-way ANOVA followed by Tukey test. In the VEH group, sepsis resulted in elevated TNF-α at 4 (934 ± 453) and 24 hours (1287 ± 437). PNT resulted in attenuation of this response at both 4 and 24 hours after sepsis induction (592 ± 62 and 671 ± 175, respectively). SPT amplified the response at 24 hours (2479 ± 875), but not at 4 hours (1167 ± 428). The results indicate that preventing endogenous ADO degradation with PNT diminishes the *in vivo* TNF-α response to sepsis, while blockade of ADO receptors amplifies this response. These data are consistent with the hypothesis that manipulating endogenous adenosine during sepsis can be used to effectively modulate serum TNF-α concentrations. Because these approaches modulate rather than completely ablate the TNF-α

response to sepsis, modification of adenosine pathways may prove a useful tool in the management of sepsis.
Supported by NIH grant GM 48219, VA Merit Review, and the Living Institute for Surgical Studies. PNT was a generous gift of SuperGen, Inc.

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cAMP INHIBITS TNFα PRODUCTION THROUGH A POST-TRANSCRIPTIONAL MECHANISM INDEPENDENT OF IL-10

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cAMP inhibits LPS stimulated TNFα production and increases IL-10. We previously found that IL-10 inhibits NFκB in human monocytes causing a decrease in TNFα release. We **hypothesized** that cAMP would decrease TNFα production through an IL-10 dependent inhibition of NFκB. **Methods:** Human monocytes were stimulated with LPS (100 ng/ml) with and without dibutyryl cAMP (100 μM), forskolin (50 μM), or IL-10 neutralizing Ab (0.1 μg/ml). Cytokines were measured by ELISA. NF-κB activity was assessed by gel shift and TNFα mRNA by RT-PCR. Statistical significance was determined by paired Student's t-test. **Results:** cAMP decreased LPS stimulated TNFα release while increasing LPS-stimulated IL-10 release. LPS-induced NFκB activation and TNFα mRNA levels were not inhibited by cAMP. Neutralization of IL-10 bioactivity (Ab) did not affect cAMP inhibition of TNFα release. Similar results were obtained when forskolin was used to increase cAMP levels (data not shown).

Condition	TNFα *(ng/ml)*	IL-10 *(pg/ml)*	NFκB *(relative density)*	TNFα mRNA *(relative density)*
Ctrl	0.1±0.04	6.8±2.8	1	1
LPS	1.6±0.3*	37.7±19.1*	2.1*	3.8*
LPS+cAMP	0.8±0.2‡	83.2±27.9‡	1.9*	4.9*
LPS+cAMP+Ab	0.9±0.1‡	--	--	--

TNFα and IL-10 presented as mean ± SE, NFκB activation and TNFα mRNA levels as relative band density, n=6 donors per group.
** P<0.05 vs. Ctrl, ‡ vs. LPS*

Conclusion: cAMP decreases LPS stimulated TNFα protein release independent of NF-κB activation or TNFα mRNA induction. Neutralization of IL-10 did not effect the ability of cAMP to inhibit TNFα. We conclude that cAMP inhibits LPS stimulated TNFα production through a post-transcriptional mechanism which is independent of IL-10.

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THE RESISTANCE OF INTERFERON-γ (INF-γ) DEFICIENT MICE TO PROPIONIBACTERIUM ACNES (PA) BACTERIAL PRIMED LOW DOSE LPS INJURY.

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PA hypersensitizes to subsequent LPS. To examine the mechanism of this injury, the influence of LPS dose and PA was evaluated in C57BL/6 and INF-γ knockout (GKO) mice.
Methods: Heat-killed PA (0.5mg/mouse) was injected 7 days before IV low dose LPS (20μg/mouse) or LPS (20 or 800μg/mouse) alone to C57BL/6 and GKO (C57BL/6 background).
Results: Survival after 20μg LPS alone, 20μg LPS+PA or 800μg LPS alone in C57BL/6 was 100%, 0% and 0%, respectively. In contrast, GKO survival was 100% in both 20μg LPS and 20μg LPS + PA but 0% with 800μg LPS. Splenectomy in C57BL/6 mice 24 hours before LPS, but not before PA, increased survival from 0% to 50%. With 20μg LPS+PA, peak plasma cytokine levels after LPS administration were significantly higher in C57BL/6 than in GKO mice, but with 800μg LPS alone, TNF-α and IL-6 levels and mortality in GKO mice were equal to C57BL/6.

Peak	Treatment	C57BL/6 (pg/ml)	GKO (pg/ml)
INF-γ	20μg LPS+PA	156 ± 15	0
(6 hrs)	800μg LPS	17 ± 5	0
(ng/ml)	20μg LPS	0.6 ± 0.1	0
TNF-α	20μg LPS+PA	306 ± 33	20 ± 1
(1 hr)	800μg LPS	113 ± 14	119 ± 33
(ng/ml)	20μg LPS	8 ± 0.1	8 ± 1
IL-6	20μg LPS+PA	400 ± 61	49 ± 4
(6 hrs)	800μg LPS	177 ± 15	146 ± 7
(ng/ml)	20μg LPS	22 ± 1	22 ± 2
IL-12	20μg LPS+PA	290 ± 108	55 ± 33
(6 hrs)	800μg LPS	102 ± 37	82 ± 20
(pg/ml)	20μg LPS	0	0

Conclusion: Lack of LPS sensitivity in interferon-γ deficient knockout mice after prior macrophage activation with PA was overcome with higher dose LPS with TNF-α levels and mortality comparable to that of normal mice.

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PATTERN OF PROINFLAMMATORY CYTOKINES IN PATIENTS WITH PURULENT-SEPTIC COMPLICATIONS OF SEVERE PANCREATITIS.

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Proinflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF, play an important role in the development and progression of acute pancreatitis. The gut is the main source of bacteria, translocated to the necrotic foci, and caused its putrification. The pattern of these cytokines was studied in 30 patients with acute pancreatitis and 12 of them had the purulent-septic complications. The cytokines level was determined by ELISA. Already, at the first day after admission the elevation of all cytokines was noted. However, the significantly highest levels of proinflammatory cytokines were observed in patients with the purulent-septic complications (p=0.01). TNF changes the metabolism in the intestinal wall, promotes its ulceration and translocation of bacteria. IL-1 has the procoagulant activity, what leads to the disorders of the local circulation and promotes to the translocation also. IL-8 involves in the neutrophil-mediated damage of the vascular wall. IL-8 also stimulates the degranulation of neutrohpils with liberation of myeloperoxydase, elastase and glucoronidase. The IL-6 has the similar effects to the IL-1 and TNF. Thus, all of these cytokines, have abilities to mediate immune mechanisms underlying intestinal inflammation. The including in the complex management the drugs with the anticytokines activity (pentoxifylline) prevent the uncontrolled liberation of proinflammatory cytokines, improves the microcirculation in the intestinal wall and, accordingly, may prevent the translocation bacteria.

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NIMODIPINE AND GLYCINE PREVENT POSTBURN SERUM-STIMULATED SECRETION OF TUMOR NECROSIS FACTOR BY KUPFFER CELLS.

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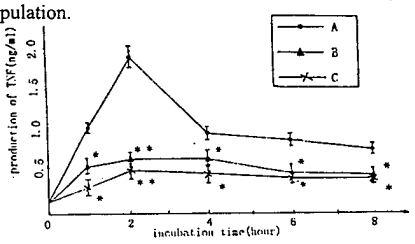
Kupffer cells (KCs) are an important component of the inflammatory response to burn injury; postburn increases in

TNF secretion by KCs have been described and alterations in [Ca^{2+}] have been hypothesized to regulate this secretory process. In addition, previous studies have suggested that KCs contain a glycine-gated chloride channel by which glycine alters [Ca^{2+}]. **METHODS**: Blood samples were collected from Sprague-Dawley (SD) rats before and 1, 2, 4, 6, 8 hours after a 3° scald burn comprising 30% TBSA. After centrifugation of blood samples, serum was prepared and stored at 56°C for 30 minutes to inactivate complement. KCs were harvested from the liver of SD rats and cultured in media alone or in media containing 10% burn serum (BS), or BS plus either nimodipine (a Ca^{2+} channel blocker, 1μM) or glycine (1mM); TNF levels were determined (ELISA) in super-natant. **RESULTS**: BS stimulation of KCs increased supernatant TNF levels (10-fold, Figure: solid lines, BS; triangles, BS+ glycine; cross, BS+ nimodipine, *p<0.05). Both nimodipine and glycine blunted BS-mediated KCs TNF secretion. **CONCLUSION**: (1) BS increases Kupffer cell TNF secretion. (2) This response is blunted by either nimodipine or glycine. (3) These data suggest that [Ca^{2+}] regulates cytokine secretion by this cell population.



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STRAIN AND GENDER VARIABILITY IN SPLENIC CYTOKINE RESPONSES FOLLOWING BURN INJURY

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Susceptibility to shock and trauma is variable dependent upon various factors, including age, gender and a genetic background that predisposes an injured subject to an exaggerated inflammatory response. The aim of this study was to examine the influence of gender and strain difference on cytokine response following burn injury. **Methods:** BALB/c and C3H/HEN 8-9wk old male and female mice (n=5/gp) were given a 20% TBSA injury. Spleens from control or injured mice were harvested on postburn day 5 and placed in serum-free media. Supernatants from unactivated or LPS (0.1ng, 1ng, 10ng, 100ng, 1ug/ml) activated splenocyte cultures were assayed for IL-1B, IL-6, IL-10 and IFNγ by ELISA. **Results:** *Gender difference:* The strongest gender difference in cytokine production was observed between male and female C3H mice. At all LPS concentrations, cells from burned males produced significantly more of all tested cytokines, as well as higher cytokine levels in the male control group for IL-10 and IFNγ($p<0.01$). In contrast, BALB/c male and female responses were equivalent, except that cells from burned females produced more IL-10, while female control and burn groups had higher levels of IL-1B and IL-6 ($p<0.04$).

Strain difference: Splenocytes from injured Balb/c males produced greater levels of IL-1B and IL-10, whereas the C3H male group had higher levels of IL-6 and IFNγ($p<0.02$). Control and burned BALB/c female groups had higher levels of IL-1B, IL-6; and IFNγ($p<0.01$), while the burned C3H female group had higher levels of IL-10 ($p<0.0001$). **Conclusions:** These results support gender differences in response to injury, with cells from C3H males producing significantly greater amounts of cytokines than cells from C3H females. However, this gender disparity in cytokine response was strain specific, as shown by the predominately equivalent response between the BALB/c males and females.

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SUBSTANCE P ACTIVATION OF THE PRO INFLAMMATORY CYTOKINE TNFα.

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Pain is the first perception of severe traumatic injury but the extent to which pain contributes to systemic shock is unknown. We studied the role of tachykinins, the nociceptive neuropeptides produced by the sensory afferent nerve endings, in regulating the production of those proinflammatory cytokines known to be involved in shock. Mice were injected with lipopolysaccharide and transcription of TNFα, Il-1 and Il-6 were analyzed by RT/PCR. Serum levels were determined by Elisa.. Tachykinin release was abolished by pretreating the mice with capsaicin. Capsaicin pretreatment impaired TNFα transcription and reduced TNFα titers while the responses of Il-1 and Il-6 are unaffected. Pretreatment with SR140333,. an antagonist for substance P, also impaired the TNFα response while blockade of the tachykinins neurokinin A and B had not effect. These findings indicate that the nociceptive signals mediated by substance P may play an important role in the induction of cytokines known to be involved in the shock response.

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COMPARISON OF ENDOTOXIN AND CYTOKINES IN PORTAL VEIN AND PERIPFERAL ARTERY.

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Background and Objective: The gastrointestinal tract is thought to motor of multiple organ failure via bacterial translocation. Here we investigated microorganism's products and cytokines in portal vein and peripheral artery. Patients & Methods: Twenty-four consecutive patients whom portal vein catheter was inserted at the elective operation for the cancer of bile duct (n=10), pancreas (n= 8) or liver (n=5) were enrolled in this study. Blood samples were collected from portal vein (PV) and peripheral artery (A) at the same time on postoperative day (POD) 1-3. Anaerobic and aerobic blood culture, endotoxin and β glycan in plasma, and TNFα, IL-6 and IL-8 in serum of PV and A were examined. Results: All blood culture was negative. No significant difference of endotoxin, β glycan, TNFα, and IL-8 existed between PV and A, except IL-6 on POD 0. Conclusions: Bacterial translocation and cytokine production from gastrointestinal tract occur in rare cases except caused by surgical maneuvers.

Table 1. Plasma endotoxin, β glycan, and serum TNFα, IL-6 and IL-8 in portal vein and peripheral artery on postoperative day 0. mean±SE (pg/ml), * P=0.0051 vs peripheral artery

	endotoxin	β glycan	TNFα	IL-6	IL-8
portal vein	6.4 ±1.7	14.8 ±4.5	1.2 ±1.2	247.9* ±38.8	158.5 ±41.2
peripheral artery	8.7 ±3.6	14.0 ±4.0	0 ±0	213.3 ±40.0	122.7 ±35.4

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ENDOGENOUS INTERLEUKIN-10 ATTENUATES SPLANCHNIC ISCHEMIA-REPERFUSION INJURY IN MICE. Z. Yang*, H.R. Wong*, Szabó C., B. Zingarelli. Critical Care Medicine, Children's Hospital Medical Center, Cincinnati, OH 45229.

It has been demonstrated that interleukin-10 (IL-10) can down-regulate the expression and release of several pro-inflammatory cytokines. In the present study we investigated whether genetic ablation of this cytokine may affect the inflammatory response after splanchnic ischemia and reperfusion. To this aim, ischemia and reperfusion injury was induced in IL-10 deficient mice ($IL\text{-}10^{-/-}$) and wild-type controls by occlusion for 45 minutes of the left superior mesenteric artery followed by 45 minutes reperfusion. In wild-type mice, ischemia and reperfusion caused splanchnic injury characterized by epithelial hemorrhagic necrosis, upregulation of ICAM-1, neutrophil infiltration (indicated by myeloperoxidase activity, 17.0±4.2 U/g tissue) and lipid peroxidation (indicated by tissue malondialdehyde, 0.7±0.1 μM). The tissue damage was associated with high plasma levels of the pro-inflammatory cytokines tumor necrosis factor (TNFα, 13.0±3.6 pg/ml) and interleukin-6 (IL-6, 217.8±85.2 pg/ml) and with high levels of the anti-inflammatory cytokine IL-10 (272.4±89.1 pg/ml). $IL\text{-}10^{-/-}$ mice subjected to splanchnic ischemia and reperfusion experienced a more severe tissue injury when compared to wild-type mice. Upregulation of ICAM-1, myeloperoxidase activity (27.5±3.6 U/g tissue) and malondialdehyde (1.3±0.4 μM) in the splanchnic tissue were significantly higher in $IL\text{-}10^{-/-}$ mice than in wild-type littermates ($p<0.05$). Plasma levels of TNFα (21.5±6.7 pg/ml) and IL-6 (429.9±18.2 pg/ml) were also greatly enhanced in comparison to the plasma levels of wild-type mice ($p<0.05$). These data demonstrate that endogenous IL-10 may exert an anti-inflammatory role during reperfusion injury, possibly by regulating ICAM-1 expression, neutrophil recruitment, and the subsequent cytokine and oxidant generation.

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EXTRACELLULAR REGULATED KINASE (ERK) AND P38: CROSS-TALK AND THE ROLE OF PHOSPHATASES. S. Arbabi*, M. Rosengart*, I. Garcia*, and R. Maier. U. of Washington, Seattle, WA 98195.

ERK and p38, two members of the mitogen activated protein kinase (MAPK) family, are known to play a central role in mediating intracellular signal transduction that affect cell proliferation, differentiation, and inflammatory cytokine production. ERK and p38 are activated by dual phosphorylation on adjacent threonine and tyrosine residues; however, the endogenous control mechanisms are not known. To elucidate these as potential therapeutic sites, we studied p38 and ERK activation in response to endotoxin (LPS) and osmotic stress (OST) in endothelial cells. **Methods**: Human umbilical vein endothelial cells were treated with LPS or increasing concentrations of NaCl. Total cell lysates were subjected to Western blot analysis using dual phospho-specific ERK1/2, p38, and MEK1/2 (ERK upstream kinase) antibodies. **Results:** LPS and OST activated both ERK and p38 with maximal activation at 30 minutes and a return to baseline in two hours. As expected, activation of MEK preceded the ERK activation. PD98059, a MEK inhibitor, inhibited ERK phosphorylation but had no effect on p38. However, SB202190 (which binds to the activated form of p38 and inhibits its function) increased the intensity and the duration of p38 and ERK phosphorylation, in response to LPS and OST, by a factor of 2.5 with no effect on MEK. Cyclohexamide, an inhibitor of de novo protein synthesis, abrogated this effect. **Conclusion:** There is a cross talk phenomenon between ERK and p38; p38 activation has an inhibitory affect on ERK phosphorylation with no effect on the ERK upstream kinase, MEK. Furthermore, p38 activation has a negative feedback on itself. These two effects require synthesis of an early gene protein. We propose that LPS and OST activate p38 which subsequently induces increased levels of at least one phosphatase which in turn down regulates ERK and p38. Since this phosphatase(s) appears to be an early gene product and acts on ERK and p38 but not MEK, the probable candidate is a dual specific MAPK phosphatase, such as PAC-1 or MAPK phosphatase-1.

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$P59^{fyn}$ AND Ca^{2+} SIGNALING IN INTESTINAL MUCOSAL T CELLS OF RATS WITH BURN OR BURN- INFECTION INJURY. M. Choudhry, A. Kohn*, T Ravindranath, A. Kambam*, S. Khan*, S. Namak*, X. Ren* and M. Sayeed. Departments of Physiology and Surgery, and Burn & Shock Trauma Institute, Loyola University Chicago, Maywood, Illinois 60153.

Our previous studies have shown that T cell proliferative disturbances in splenic T cells during sepsis could result from alterations in $P59^{fyn}$ and Ca^{2+} signaling. To investigate the role of these two signaling components in the intestinal T cells, the present study examined $P59^{fyn}$ autophosphorylation (Autophos) and its kinase activity (Enolase), and $[Ca^{2+}]_i$ in T cells harvested from Peyer's patches (PP) and mesenteric lymph nodes (MLN) following burn and burn infection. Male Sprague-Dawley rats, (250-275 g), were subjected to third degree burn (25% TBSA). Sham rats were immersed in 37 °C water. On day-1 post burn, a group of sham and burn animals were injected intraperitoneally with *E. coli* ($\sim 10^9$ CFU) . Animals were sacrificed on day-3 post burn. The results are as follows:

	PP				MLN			
	C	C+I	B	B+I	C	C+I	B	B+I
$P59^{fyn}$:								
Autophos	100	79±6$	46±4	25±9	100	45±8	66±3	60±6
Enolase	100	82±9	40±5	36±7	100	76±9	81±7	73±7
$[Ca^{2+}]_i$	152±9	89±7#	56±8	60±7	213±8	180±9	89±8	90±8

$mean (±SE), #$[Ca^{2+}]_i$=elevation over basal $[Ca^{2+}]_i$ (=125±20 nM in PP, and 110±16 in MLN).

Our data demonstrate a significant decrease in the $P59^{fyn}$ autophosphorylation and kinase activity along with attenuated $Ca^{2+}{}_i$ responses in T cells from PP of burn and burn-infected rats. Such decreases in $P59^{fyn}$ and Ca^{2+} signaling could contribute to immune functional deficits in intestinal mucosa. (Supported by NIH grants GM 53235 and GM 568501).

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DIFFERENTIAL REGULATION OF MUSCLE PROTEIN METABOLISM BY INSULIN-LIKE GROWTH FACTOR (IGF)-I AND IGF BINDING PROTEIN (BP)-1. R A. Frost and CH Lang, Penn State College Medicine, Department of Cell. Molec. Physiology, Hershey, PA 17033.

BP-1 is overexpressed during catabolic conditions, such as endotoxemia, sepsis, and burn. We have previously demonstrated that BP-1 protein increases in muscle during these conditions, and this accumulation may antagonize IGF-mediated anabolic actions leading to the observed derangements in protein balance. IGFBP-1 is a multi-functional protein that binds IGF-I in solution and to integrin receptors on the cell surface. To define a potential physiological role for BP-1 in regulating muscle protein

balance we examined the effect of IGF-I and BP-1 on protein synthesis and degradation in human skeletal muscle cells. IGF-I stimulated protein synthesis by 20%, as assessed by [^{3}H]phenylalanine incorporation, and this was completely inhibited by BP-1. Half-maximal inhibition of protein synthesis occurred at a molar ratio of BP-1 to IGF-I of 1.5:1. BP-1 failed to form a complex with a truncated form of IGF-I (desIGF-I) and consequently failed to inhibit the ability of desIGF-I to stimulate protein synthesis. Individually IGF-I and BP-1 dose-dependently inhibited protein degradation. BP-1 inhibited protein degradation, but failed to block the ability of IGF-I to do the same. Blocking integrin receptor occupancy with the integrin antagonist echistatin blunted the ability of BP-1 to inhibit protein degradation, but had no significant effect on IGF-I mediated changes in protein synthesis or degradation. The extracellular matrix protein vitronectin also inhibited protein degradation to a similar extent as BP-1, but anti-vitronectin receptor antibodies failed to inhibit the effect of BP-1 on protein degradation. Rapamycin inhibited IGF-I-dependent increases in protein synthesis, but not the ability of IGF-I to inhibit proteolysis. In contrast, rapamycin completely blocked the ability of BP-1 to inhibit proteolysis. Our results demonstrate that BP-1 inhibits protein synthesis by binding to IGF-I. BP-1, acting independently of IGF-I, also inhibits protein degradation and this response occurs via integrin receptor binding and stimulation of a rapamycin-sensitive signal transduction pathway. (Supported by NIGMS 38032).

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DILTIAZEM INHIBITION OF PMN PRIMING DURING HEMORRHAGE/RESUSCITATION. H. Jacob*, A. Pizanis*, S. Rose. Univ. Saarland, Trauma Surgery, 66421 Homburg, Germany

PMN priming and activation are pivotal mechanisms of leukocyte-derived tissue injury following ischemia/reperfusion and traumatic injury. Changes of cellular Ca^{2+} concentrations are crucial for PMN function. Aim of the present study was to study altered PMN function and Ca^{2+} regulation after hemorrhagic shock in the rat. *Methods.* Anesthetized, male Sprague-Dawley rats (~300g, n $\geq$7) were hemorrhaged to 40 mmHG for 60 min and resuscitated with citrated shed blood blood and Ringer's lactate. Diltiazem (DZ) was infused at doses of 0.1 and 0.8 mg/kg either with or 6 hrs after resuscitation. PMN were isolated during two days by Percoll gradients. PMN-superoxide production (PMN-SOP; nmol/min/10^6 cells) was determined by cytochrom C reduction, lung and liver myeloperoxidase (MPO) activity (U/g tissue) by o-dianisidine oxidation at 460 nm. Basal cytosolic Ca^{2+} concentration (nM, $[Ca^{2+}]_i$) and FMLP-induced (10^{-7} M) cytosolic Ca^{2+} mobilization were measured fluorometrically with FURA-2/AM (340/380 ratio). *Results.* DZ (0.1 or 0.8 mg/kg) significantly decreased fMLP-dependent PMN-SOP and lung MPO content after 24 hrs, when given with reperfusion ($p<.05$). DZ dose-dependently decreased basal PMN $[Ca^{2+}]_i$ (33$\pm$7 nM, 0.8 mg/kg) compared to untreated shock rats (108$\pm$20 nM)($p<.05$). *In conclusion,* hemorrhage/resuscitation caused PMN-priming with increased PMN $[Ca^{2+}]_i$ and lung PMN infiltration. DZ effectively prevented these changes when administered, even at low doses, with resuscitation. Reduction of leukocyte activation and priming could be advantagous to reduce secondary tissue damage in traumatic and ischemic injury.

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THE ROLE OF P38 MAPK IN THE REGULATION OF TNF-α GENE EXPRESSION IN RAW CELLS STIMULATED WITH LPS. Y. Jiang, L. Zhang*, A.H. Liu*, W.M. Chen* and K.S. Zhao, Key Lab. for Shock and Microcirculation of PLA and Dept. of Pathophysiology, 1st Mil. Med. Univ. Guangzhou 510515, PR China

TNF if one of the most important mediators involved in the development of endotoxic shock. To study the molecular mechanism of TNF-α expression induced by LPS, we studied the role of p38 MAPK in the gene regulation of TNF-α. In this study protein kinase assay was used to detect the kinase activity stimulated by LPS. Laser scan confocal microscopy technique was used to show the translocation of p38 on the activation. RT-PCR and luciferase reporter gene system driven by the TNF-α promoter were used to study the molecular mechanism of TNF-α gene transcription. In RAW264.7 cells it was found that p38 was activated on the stimulation of LPS, which brought about the translocation of p38 into nucleus. TNF-α mRNA increased on the stimulation of LPS and the enhanced TNF gene expression could be inhibited significantly by specific inhibitor for p38. In the experiment of co-transfection in RAW cells it was found that there was a significant relevance between the activation of p38 by LPS and the induction of TNF-α reporter gene transcription. Co-transfection of p38 with the active mutant of its upstream kinase MKK6b induced the expression of luciferase in a large amount. Further studies showed that the induction of TNF-α promoter activity by MKK6b(E) and LPS was similar. In addition, the dominant negative form of p38 or p38 inhibitor gave an inhibitory effect on the TNF-α promoter activity induced by MKK6(E) or LPS. All these results suggest p38 MAPK was activated on the stimulation of LPS, which brought about its entry to the nucleus to act on transcription factors to regulate cellular processes. p38 MAPK signal pathway was involved in the regulation of TNF-α gene expression induced by LPS.

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INHIBITION OF NF-*κ*B REDUCES APOPTOSIS IN PEYER'S PATCH B-CELLS DURING POLY-MICROBIAL SEPSIS. A.R.T. Joshi*, C.S. Chung*, G. Song*, A. Ayala, Center for Surgical Research, Brown University School of Medicine/Rhode Island Hospital, Providence, RI 02912.

Apoptosis (Ao) has been implicated as an important component of the secondary hypo-responsive part of the immune response to sepsis, which can lead to multiple organ failure. The aim of this study was to determine the role of the ubiquitous transcription factor, NF-*κ*B, in controlling the onset of apoptosis in Peyer's Patch B220 positive B-cells during sepsis. Pyrrolidine dithiocarbamate (PDTC), an NF-*κ*B inhibitor, was given to mice subjected to CLP, and the level of apoptosis in these animals' Peyer's Patch B-cells was then compared to those of the control groups. Three groups of mice were established: CLP with pre-treatment of 100mg PDTC/kg body weight in a saline vehicle (subdermal), CLP with vehicle, and Sham with vehicle. Peyer's Patches were then harvested from all mice after 24 hours. B-cells were isolated from the Peyer's Patches by homogenization and a single step 60% Percoll gradient. The cells were then standardized to 2-3 x 10^6 cells/ml and labeled with anti-B220 conjugated to R-phycoerythrin (R-PE). They were then permeabilized, and their DNA stained with a fluorescent dye (TdT-mediated dUTP nick end labeling (TUNEL)) that labels 5' ends of fragmented genomic DNA produced during

apoptosis. The cells' phenotypic changes were determined by 2-color flow cytometry.

Sham	CLP	CLP/PDTC
12.3 ± 0.96	18.7 ± 2.18	12.53 ± 1.01

The results (N=5-10) show that the administration of PDTC prior to CLP significantly decreased ($p<0.05$, T-test) the level of apoptosis (as % of total cells) in Peyer's Patch B-cells. We conclude, therefore, that NF-*k*B does play a role in the regulation of the apoptotic cascade of B-cells in the Peyer's Patches of mice subjected to polymicrobial sepsis. (NIH GM 53209).

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MOLECULAR BASES OF IMMUNOMODULATION WITH HYPERTONIC SALINE. W.G. Junger. University of California San Diego, San Diego, CA 92103.

Hemorrhagic shock provokes a rapid inflammatory response that can be followed by an anergic phase. The inflammatory phase causes excessive neutrophil activation, tissue damage, and organ failure while the subsequent anergic phase paralyses T-cell responses and renders patients susceptible to infections and sepsis.

Hypertonic saline (HS) can be used as an alternative resuscitation fluid since clinical trials have proven it safe to use. Several animal studies have shown that HS can modulate cellular immune responses to hemorrhagic shock. How could hypertonic saline affect the complex cellular responses in hemorrhagic shock?

We have found that HS activates specific signaling responses in neutrophils and T-cells. These specific signaling cascades interact with conventional signaling pathways that lead to the activation of neutrophils and T-cells. The cross-talk between the signaling pathways triggered by HS and conventional cell activation can block certain downstream signaling steps and enhance others. Depending on the receptor type through which cell activation occurs, HS can selectively block or enhance activation signaling pathways and diminish or reinforce subsequent cellular responses.

In neutrophils, G protein coupled receptor activation is blocked by. HS seemingly via the cAMP/PKA pathway. This results in suppressed neutrophil adherence, oxidative burst, and degranulation which could prevent neutrophil mediated tissue damage in trauma patients. In contrast, HS cannot block the activation of T-cells by intercepting antigen receptor signaling but co-stimulates gene transcription by activating the MAPK p38 cascade. This co-stimulates T-cell proliferation, restores the function of anergic T-cells, and may prevent infections and sepsis in trauma patients.

These studies indicate that HS could be useful to manipulate immune responses in shock by ability to differentially modulate cellular activation signaling.

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INTACT AND CLEAVED DELTA PROTEIN KINASE C (δ–PKC) ASSOCIATES WITH AND PHOSPHORYLATES THE P60 TUMOR NECROSIS FACTOR RECEPTOR IN HUMAN NEUTROPHILS. L.E. Kilpatrick, Y-H. Song*, A. Goren*, M.W. Rossi* and H.M. Korchak*. Univ. of Pennsylvania & Children's Hospital of Philadelphia, Philadelphia, PA 19104

Tumor necrosis factor (TNFα) is a potent proinflammatory cytokine that triggers oxygen radical release and degranulation in adherent neutrophils. The p60TNF receptor (p60TNFR) is responsible for proinflammatory signaling and protein kinase C (PKC) is a candidate for the regulation of p60TNFR. In the present study we determined whether p60TNFR was phosphorylated by PKC in an isotype specific manner. Activation of adherent neutrophils by TNFα, PMA, or fMet-Leu-Phe triggered p60TNFR phosphorylation that was not tyro-sine kinase mediated. Staurosporine (STAR) inhibited ligand-induced phosphorylation of p60TNFR suggesting phosphorylation is by a STAR-sensitive serine/threonine kinase such as PKC. Moreover, STAR enhanced TNFα triggered elastase release indicating that phosphorylation of p60TNFR was associated with desensitization. In resting cells, there was little or no association of p60-TNFR with different PKC isotypes. Following activation, δ–PKC and δ–PKC cleavage products associated with p60TNFR within 5 min, while β_{II}-PKC and β_{I}-PKC associated with p60TNFR following a lag period of > 5 min. Full length and cleaved δ-PKC, but not β_{II}-PKC or β_{I}–PKC, phosphorylated p60TNFR. Thus, full length and cleaved δ–PKC were the only PKC isotypes that both associated with p60-TNFR in the correct time frame and phosphorylated p60TNFR suggesting that δ–PKC acts as a negative regulator of p60TNFR and of TNFα induced signaling. (NIH Grant AI24840)

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SULFATION AND WOUND HEALING. M. D. Prager*, R. Mariappan*, E. Alas*, J. Williams*, (Spon: J. Horton). UT Southwestern, Dallas, TX 75235-9160.

Early after injury hyaluronan (HA) is prominent in granulation tissue. In the second week, HA wanes and sulfated glycosaminoglycans (GAGs) predominate. The temporal relationship between the transition from unsulfated HA to sulfated GAGs and phenotypic changes in fibroblasts in the wound bed suggest their interrelatedness. This possibility was investigated through use of chitosan (partially deacetylated poly N-acetylglucosamine) and its sulfated product as model compounds. Ability of cultured human foreskin fibroblasts (1) to bind and (2) to contract lattices of collagen alone, collagen-chitosan, and collagen-chitosan sulfate was determined. Adherence of fibroblasts, plated on a substrate lattice for 24 hr, was determined by staining with MTT and measuring A_{570}. Adherence to the collagen-chitosan latttice was markedly reduced (mean $A_{570} \pm SD = 0.16 \pm 0.05$; n=6) ($p<0.01$) compared to adherence to collagen alone ($A_{570}=0.92 \pm 0.04$) or to a collagen-chitosan sulfate lattice ($A_{570}=0.84 \pm 0.05$). Contraction of lattices containing10^6 fibroblasts enmeshed in the gel was measured planimetrically. Surface area, determined at 1-48 hr after loosening gels, permitted evaluation of the kinetics of contraction. Contraction of the collagen-chitosan gels (n=5) was less at all time points than for the other two lattices. At 48 hr the former contracted 30.0% ± 4.4 ($p<0.01$) compared to 66.9% ±4.7 for collagen alone and 71.6% ± 7.7 for collagen-chitosan sulfate lattices. Scanning electron microscopy showed the fibers of the collagen-chitosan lattice to be the thickest with altered organization. Conclusion: chitosan inhibits both fibroblast adherence to collagen and the contraction of collagen lattices, a process reversed by sulfation. Since fibroblast motility is mediated through extracellular matrix receptors, sulfation may serve as a regulatory signal.

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DILTIAZEM IN MULTIPLE TRAUMA: RESULTS OF A RANDOMIZED CLINICAL STUDY. S. Rose, G. Tosounidis*, M. Schmidt*, T. Ziegenfuss*, I. Marzi. Univ. Saarland, Trauma Surg., 66421 Homburg

Beneficial effects and reduced cellular Ca^{2+} concentrations by Ca^{2+} blocker diltiazem were reported in

various animal studies on shock, trauma and sepsis. *Methods.* Multiple traumatized patients (ISS $\geq$ 27, age: 18–65, written informed consent) were prospectively randomized and infused intravenously with either NaCl (0.9%, n=15) or diltiazem (DZ)(DILZEM®, Goedecke, Germany, n=15) over 72 hrs. Treatment begin < 12 hrs after trauma, observation during 14 days. Exclusion criteria: profound shock, limiting head trauma, specific contraindications. Daily plasma values of IL-6, IL-8, IL-10 by ELISA. Determination in isolated PMN of superoxide production (PMN-SOP; nmol/min/10^6 cells) by cytochrom C reduction and cytosolic Ca^{2+} concentration (nM, $[Ca^{2+}]_i$) fluorometrically with FURA-2/AM (340/380 ratio). Statistics: ANOVA. *Results.* Both groups were comparable in age and ISS (no DZ: 36.5 $\pm$ 7; DZ: 34.9 $\pm$ 8). DZ reduced ICU stay from 22.6 $\pm$ 12 (untreated patients) to 16.0 $\pm$ 10 days. DZ significantly decreased both fMLP-dependent PMN-SOP and basal $[Ca^{2+}]_i$ observed in untreated patients 24 hours after trauma. In DZ-treated patients, lower IL-6 and CRP concentrations were observed. *Conclusion.* DZ infusion in resuscitated trauma patients did not produce any adverse effects, nor hypotension or arrhythmias and was safe. The significant attenuation of priming and cytosolic Ca^{2+} concentrations of circulating PMN of DZ-treated patients suggested anti-inflammatory effects, which were reflected by lower IL-6 and CRP concentrations. Immunomodulation of severe injury by diltiazem is a promising approach for future clinical studies.

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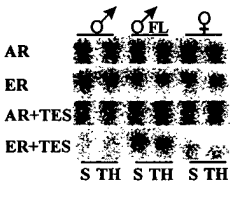
ANDROGEN-DEPENDENT CHANGES IN ESTROGEN RECEPTOR EXPRESSION (ER) AND IL-6 RELEASE BY SPLENIC T LYMPHOCYTES FOLLOWING TRAUMA-HEMORRHAGE (TH). T.S.A. Samy*, M.G. Schwacha, W.G. Cioffi, K.I. Bland* and I.H. Chaudry. Center for Surgical Research and Dept of Surg., Brown Univ. Sch. Med., Rhode Island Hospital, Providence, RI 02903.

Sex steroids, testosterone (TES) and β-estradiol, play a major role in inflammatory processes since they induce transcription of several cytokine genes by interaction with their intracellular receptors. A profound immunosuppression with typical loss in T lymphocyte functions after TH is seen in males but not in females. Thus, our aim was to determine whether: (i) receptors for androgen (AR) and ER are present in splenic T lymphocytes, (ii) expressions of AR and ER are altered following TH and (iii) TES receptor antagonism alters AR and ER expressions and IL-6 release. Our study demonstrated the presence AR and ER in nuclear extracts of mouse splenic T lymphocytes by three independent assays: electrophoretic mobility shift assay (EMSA), Western immunoblot and ^{14}C-labeled TES or estradiol binding. Neither gender, TH nor Flutamide (FL, androgen antagonist) treatment of males influenced expressions of AR or ER in T lymphocytes (Fig. EMSA). TES stimulation of T lymphocytes *in vitro*, however, enhanced expression of ER but not AR only in cells from FL-treated sham and TH. IL-6 release by T cells from males following TH showed TES dependency for the activity. These results suggest that the anti-androgenic effect of FL is not mediated through AR alone and other mechanisms entailing TES regulation may be involved. Furthermore, TES enhanced ER expression in T lymphocytes of Flutamide-treated males indicates that anti-androgens are capable of modulating the estrogen function at the receptor level. (Supported by NIH grant GM37127).



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PICROLIV ACTIVATES MITOGEN ACTIVATED PROTEIN KINASES (MAPK) AND INDUCES HEAT SHOCK PROTEINS DURING ISCHEMIC-REPERFUSION INJURY. G. S. Sidhu*, N. Goyal*, P. Seth*, H. Mani* S. K. Sharma*[1], D. K. Kulshreshtha*[1] and R. K. Maheshwari*, (Spon: P. Rhee) *Centre for Combat Casualty Care and Life Sustainment Research, Department of Pathology, USUHS, Bethesda, MD 20814 and [1]CDRI, Lucknow, India.

Preconditioning is a critical factor to overcome ischemic damage. We examined the expression of heat shock proteins (HSP70 and HSP90), and MAPK(ERK1 and ERK2) to investigate the molecular mechanism of protection of picroliv, derived from a plant, *Picrorhiza kurrooa* against hypoxia, and ischemia-reperfusion injury (IRI) in rats. Picroliv(12mg/Kg) was fed daily to male Sprague Dawley rats by oral gavage for 7 days prior to IRI or hypoxic stress. Hepatic ischemia was induced by occluding the hepatic pedicle with microaneurysm clip for 30 min, followed by reperfusion for varying periods (15-120 min). For hypoxic stress, animals were exposed to 10% oxygen for four days. The localization of HSPs was examined using monoclonal Ab against the inducible form of HSP70 and HSP90 by immunohistochemistry. Both HSP70 and HSP90 were induced in IR or hypoxic injury, however, picroliv further enhanced the expression of these proteins in liver and intestine. Picroliv treatment activated phosphorylated form of ERK1 in various organs as revealed by immunohistochemistry Western blot analysis showed that pre-treatment of picroliv induced the expression of HSP70 and HSP90 in HUVEC and NIH3T3 cells exposed to hypoxia (1% oxygen). This induction was mediated by the transactivation of the heat shock transcription factor-1(HSF-1) as revealed by DNA binding studies. Confocal microscopy, and Western blot studies revealed the activation of MAPK in picroliv pre-treated renal epithelial (NRK52E) cells exposed to chemical hypoxia. These results suggest that picroliv protects against hypoxic/ischemic injury through signaling cascades that induce the transcription of genes such as HSPs.(Supported by ONR grant G174HV and G174GV).

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ROLE OF PROTEIN TYROSINE PHOSPHATASES IN TGF-β-MEDIATED INHIBITION OF SRC-KINASES.
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Departments of Physiology and Surgery, and Burn & Shock Trauma Institute, Loyola University Chicago, Maywood, Illinois 60153.

TGF-β has been shown to suppress T cell activation and proliferation. In our recent studies we found that such suppression of T cell proliferation could result from a decrease in the phosphorylation and kinase activity of src-related protein tyrosine kinases, $P59^{fyn}$ and $P56^{lck}$. The present study examined the role of Protein tyrosine phosphatases (PTP) in TGF-β-mediated suppression of $P59^{fyn}$ and $P56^{lck}$ autophosphorylation (Autophos) and their kinase activity (Enolase). Splenic T cells from Sprague Dawley rats were pretreated with TGF-β (25 ng/ml) and thereafter stimulated with either anti-CD3

or inhibitor of PTPs, sodium pervanadate (PV). The results are as follows:

	anti-CD3 (%inhibition)		PV (% inhibition)	
	$P56^{lck}$	$P59^{fyn}$	$P56^{lck}$	$P59^{fyn}$
Autophos	60#	70	7	11
Enolase	50	45	5	6

#, % inhibition of control (100%)

In conclusion, these result suggest that TGF-β may suppress $P56^{lck}$ and $P59^{fyn}$ via up-regulating the protein tyrosine phosphatases.(Supported by NIH grants GM 53235 and GM 568501).

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IRAK PARTICIPATES DIRECTLY IN LPS SIGNAL TRANSDUCTION. J.L. Swantek*, M. Tsen*, M.H. Cobb*, and J.A. Thomas*, (Spon: B. Giroir). Univ. Texas Southwestern Medical Center, Dallas, TX 75235.

LPS, a common component of the gram-negative bacterial outer wall, precipitates many of the hemodynamic, hematologic and inflammatory perturbations seen in clinical septic shock. Macrophages exposed to endotoxin respond by secreting several pro- and anti-inflammatory mediators and cytokines. Recent studies demonstrate that the Toll-like receptor 4 (Tlr4) is the signaling endotoxin receptor. Tlr4 belongs to the Toll/IL-1 receptor family and transfection studies suggest that Tlr4 signals via a conserved intracellular signal transduction pathway, which includes MyD88, the interleukin-1 receptor associated kinase (IRAK), and the TNF receptor associated factor 6. We have knocked out the IRAK gene in mice and demonstrate pronounced impairment of LPS signaling in IRAK-deficient macrophages. Thioglycollate-elicited peritoneal macrophages from knockout (KO) mice produce significantly less TNF-α in response to LPS than cells from wild-type mice. This difference occurs over a 10-fold dose range of LPS (LCD 25, 0.2-2 ng/ml). IRAK-deficient macrophages exhibit perturbed signal transduction down several intracellular kinase cascades. LPS-induced JNK/SAPK activity, as well as activity of one of its upstream activators, MEK4, is attenuated in KO cells. LPS-induced activation of I kappa B kinase-α (IKK-α), which catalyzes the reaction leading to activation and nuclear translocation of NF-κB, a potent transcription factor mediating the acute inflammatory response, is severely reduced in KO cells. This reduction of IKK-α activity is reflected in decreased NF-κB DNA binding activity. Our results indicate a critical role for IRAK in LPS triggered responses.

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THE ROLE OF NF-κB IN THE PROTECTIVE EFFECT OF HEAT SHOCK RESPONSE AGAINST CARDIOMYOCYTES INJURY INDUCED BY HYDROGEN PEROXIDE. Weimin Xiao*, Lin Zhong*, Jialu You* and Zhengyao Luo*,(Spon:Shilin He). Dept. Of Pathophysiology, Hunan Medical University, Changsha, Hunan, 410078 P.R.China

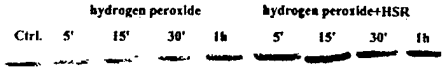
In previous study, we have demonstrated that heat shock response (HSR) could protect against cardiomyocytes injury induced by hydrogen peroxide (H_2O_2) in vitro [Shock 1994,1(suppl):A201], but its mechanism remains unclear. There are growing evidences showing that the protective effects of HSR are related to the inhibition of activity of NF-κB by I-κBα (an endogenous inhibitory protein of NF-κB). The primary cultured neonatal rat cardiomyocytes were Exposed to H_2O_2 (1mmol/L, 3h) 6h after HSR(43℃,1h). Results showed that HSR significantly protected cardiomyocytes against H_2O_2-induced injury (Tab.1). In addition, Western blot analysis revealed that: ①HSR could induce expression of heat shock protein-70KD (HSP70), and ②HSR could prevent H_2O_2-induced degradation of I-κBα (Fig.1). Immunohistochemistry revealed that HSR could block H_2O_2-induced translocation of NF-κB from cytoplasm to nucleus. These data indicated that HSR-mediated protection against H_2O_2-induced myocardial cell injury might be conferred by the expression of HSP70 which could inhibit NF-κB activity.

Tab1.Effect of HSR on H_2O_2-induced myocardial cell injury

group	n	release rate of LDH (%)	cell death rate (%)
control	6	4.73±1.09	15.2±3.0
H_2O_2	6	58.95±3.86*	55.7±4.3*
HSR+H_2O_2	5	45.65±2.54#	41.6±3.6#

* vs control , P<0.01; # vs H_2O_2, P<0.01. value = $\bar{x}$ ±s

Fig.1 Western blot showed the presence of I-kBa in cardiomyocytes.



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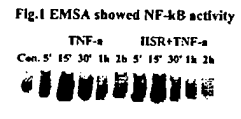
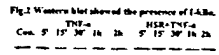
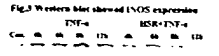
INHIBITION OF NF-κB ACTIVITY MIGHT CONTRIBUTE TO THE PROTECTIVE EFFECT OF HEAT SHOCK RESPONSE ON TNF-α -INDUCED ENDOTHELIAL CELL APOPTOSIS. Jialu You*Lin Zhong*, Weimin Xiao*, Meidong Lui*, and Zhengyao Luo*,(Spon:Shilin He). Dept. Of Pathophysiology, Hunan Medical University, Changsha, Hunan, 410078 P.R.China

We have previously reported that TNF-α induces apoptosis in cultured bovine pulmonary artery endothelial cells (BPAEC) via NO produced by inducible nitric oxide synthase (iNOS) resulting from the activation of NF-κB [Shock 1998,1(suppl):A33]. The heat-shock response (HSR) is a highly conserved cellular stress response affording cytoprotection against a variety of cytotoxic conditions. Recently there are growing evidences showing that the protective effects of HSR are related to inhibition of proinflammatory gene expression by blocking I-κBα (an endogenous inhibitor of NF-κB) degradation and NF-κB nuclear translocation. In this study, we investigated whether HSR could prevent BPAEC from TNF-α -mediated apoptosis and its mechanism related to modulating NF-κB activity. BPAEC were exposed to TNF-α (2500U/ml,24h) 16h after the treatment of HSR(43℃,1h). The apoptotic morphological changes, DNA ladder pattern on agarose gel electrophoresis, and percentage of DNA fragmentation of BPAEC were analyzed. The results showed that HSR induced expression of heat-shock protein-70KD (HSP70) and significantly attenuated TNF-α -induced apoptosis in BPAEC. Further, electrophoretic mobility shift assays(EMSA) revealed that HSR inhibited TNF-α -mediated NF-κB activity (Fig.1). Western blot analysis revealed that HSR also inhibited TNF-α mediated I-κBα degradation(Fig.2) and iNOS expression(Fig.3). These data indicated that HSR could protect BPAEC against TNF-α -induced apoptosis, and its mechanism might involve HSP70 inhibition of TNF-α -mediated NF-κB activity and iNOS expression.

Fig.1 EMSA showed NF-kB activity







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EFFECT OF CHRONIC PERITONEAL SEPSIS ON MORTALITY IN FEMALE RATS HB Bosmann[1], V Prisk[1]*, G Choudhary[1,3]*, AC Sharma[1], WR Law[1,2] and JL Ferguson[1], Departments of Physiology & Biophysics[1], Surgery[2], and Michael Reese Hospital[3], The University of Illinois at Chicago, College of Medicine, Chicago, IL.

In the present study, we hypothesized that the phase of the ovarian cycle at the time of induction of sepsis in female rats would affect the mortality. Female rats (300-350 g) were randomized to septic and non-septic groups. Sepsis was induced with an ip injection of a cecal slurry obtained from donor rat (200 mg/kg in 5 ml 5% dextrose in water (D_5W); ip), while non-septic rats received only sterile D_5W. A group of septic male rats (age matched) was included to compare the mortality rate obtained in septic male vs septic female groups. Prior to the induction of sepsis, a vaginal smear was analyzed to confirm the ovarian cycle phase of the female rats. The mortality endpoint was seven days after induction of sepsis. After seven days, blood was collected from surviving rats to measure serum levels of steroid hormones. Preliminary data showed that male septic rats had a 57 % mortality.

7 day post-sepsis	Septic Male	Septic Females		
		Proestrus	Estrus	Diestrus
dead/total	4/7	1/4	3/3	2/9

Female septic rats had a reduced mortality rate during proestrus (25%), and diestrus (22%). An increased mortality during the estrus (100%) phase of their ovarian cycle (Table above) is comparable with previously reported increases in serum estradiol levels in septic male rats. Thus in the present study, lethality due to sepsis was highly dependent on the stage of the estrus cycle in the female rats and may be attributable to altered circulating steroid hormone levels.

[This work is funded by AHA Senior fellowship (ACS) & RO1 GM 48219 (JLF & WRL)]

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DISCREPANCIES BETWEEN EXPERIMENTAL AND NATURALLY OCCURRING SEPSIS IN CATS.

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Introduction: Experimental models of sepsis have been criticized as clinically irrelevant. The failure of these models to translate to the clinic may be a function of the model or the species. **Methods**: Hemodynamic, hematologic, and pathologic data were collected from records of 30 cats with naturally occurring severe sepsis confirmed by necropsy, and from data reported in feline experimental models of sepsis. **Results**: Naturally occurring sepsis in cats consistently resulted in hypotension, bradycardia, hypothermia, leukocytosis, thrombocytopenia, and anemia. Leukopenia and gastrointestinal (GI) injury were rare. Experimentally induced sepsis in cats typically resulted in hypotension, tachycardia, variable changes in core temperature, increased hematocrit, leukopenia, and GI injury. **Conclusions**: Feline experimental models of sepsis do not mimic the findings in naturally occurring feline sepsis. These results suggest that even when interspecies differences are eliminated, experimental models of sepsis do not accurately reproduce some features of the clinical syndrome.

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HIGH DOSE OF INTRAPERITONEAL L-ARGININE AND MULTI-ORGAN FAILURE IN SHEEP. H. Ho, S. Griffey*, K. Cochrum* and R. Gunther. Dept. of Surgery, Sch. of Med. and Animal Resources Service, Sch. of Vet. Med., Univ. of Calif., Davis, 95616.

L-arginine in high doses causes pancreatic injury in rodents with the development of acute pancreatitis. In the present study, we investigated the relation between high dose intraperitoneal L-arginine and both pancreatic and renal function in sheep. Preliminary studies suggested direct injury to the kidney in this species. Both hemodynamic parameters and renal function following L-arginine were monitored. Sheep were prepared with vascular catheters in both the carotid artery and jugular vein and a foley bladder catheters was utilized to monitor renal function. Vascular pressures, cardiac output, and renal function were monitored for 3 days following the administration of 3.0 gm/kg to 3.75 gm/kg of L-arginine into the abdominal cavity. One hour after instillation, monitoring was initiated. Fluid balance was determined as the difference of fluids given and total urinary output. Histological evidence of pancreatic and renal pathology was present. Renal findings included diffuse acute tubular necrosis associated with oliguria. Mean fluid balance after 3 days was 5.5 liters. We conclude that a high dose of intraperitoneal L-arginine damages not only the pancreas but the kidney in sheep.

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$rBPI_{21}$ IN A CLINICALLY RELEVANT MODEL OF SEPSIS CB Jackson*, E Rogell*, R Prior*[$], S Ammons*[#], CM Otto,. Sch of Vet Med, Univ. of Pennsylvania, Philadelphia, PA 19104; [$]The Ohio State Univ, Columbus, OH 43210, [#]XOMA Corp., Berkeley CA

Canine parvovirus (CPV) leads to intestinal injury, bacterial translocation, endotoxemia, sepsis, and death. CPV represents a clinically relevant animal model to study the treatment of human sepsis. $rBPI_{21}$, a modified recombinant N-terminal fragment of human bactericidal/permeability-increasing protein with antimicrobial and endotoxin neutralizing activities, has demonstrated benefit in experimental models of sepsis and in meningococcemia patients. **METHODS:** Randomized, double-blind, placebo-controlled clinical trial. Forty dogs with clinical signs, CPV fecal antigen, client consent and no prior treatment were enrolled. $rBPI_{21}$ or placebo was infused over 6 hours. Vitals were recorded and monitored. Plasma endotoxin levels were measured at 0, 3 and 6 hours and $\geq$ 4 weeks using a quantitative chromogenic Limulus Amebocyte Lysate test.

Survival was determined at 1 month. **RESULTS:** Overall survival rate was 82.5%; BPI 90% vs placebo 75% (p=0.249). Initial endotoxin levels (mean 21.0 pg/ml ± 10.6) were significantly higher than values in recovered dogs at ≥ 4 weeks post infection (mean 11.4 pg/ml ± 7.6), (p=0.0019). No significant difference in endotoxin levels occurred between the two groups at any time point. **INTERPRETATION:** Dogs infected with CPV represent a naturally occurring, reproducible model where viremia leads to endotoxemia, and sepsis, and may provide a valuable link between experimental animal models and human clinical trials. Though there was a numerical advantage in the $rBPI_{21}$ group, additional studies are warranted.

Supported by Bernice Barbour Foundation & XOMA Corp

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GRAM POSITIVE CELL WALL COMPONENTS PROMOTES GUT BARRIER FAILURE AND BACTERIAL TRANSLOCATION. E. Nadler*, X. Zhou*, J. Upperman*, S. Alber*, S. Watkins*,H. Ford. Children's Hospital of Pittsburgh, Pittsburgh PA, 15213

Introduction: We have previously shown that endotoxin promotes gut barrier failure and bacterial translocation. Yet, the mechanism by which gram-positive organisms induce sepsis remains unclear. The gram-positive bacterial cell wall components peptido-glycan G (pep G) and lipoteichoic acid (LTA) can induce the systemic inflammatory response syndrome, however their effect on gut barrier function and BT is unknown.

Methods: Sprague-Dawley rats received one of three dosing regimens of pep G and LTA intravenously. Group 1: 3 mg/kg LTA and 10 mg/kg pep G, Group 2: 3 mg/kg LTA and 30 mg/kg pep G, Group 3: 10 mg/kg LTA and 30 mg/kg pep G. Blood, mesenteric lymph nodes, liver, and spleen were cultured after 24 h for translocation.

Results: Two of 3 rats in each treatment group translocated. *E. coli* was the predominant translocating organsim. Immuno-histochemistry revealed an increased incidence of enterocyte apoptosis and 3-nitortyrosine residues in the intestinal mucosa of rats in Groups 2 and 3.

Conclusion: Our preliminary data show that pep G and LTA promote gut barrier failure and BT by inducing enterocyte apoptosis. The mechanism may involve increased nitric oxide production and subsequent nitration of proteins in the intestinal mucosa. The results suggest that gut barrier failure also occurs in gram-positive sepsis.

Group	BT	AST	OCT
1	2/3	NA	NA
2	2/3	306 ± 261	45.5 ± 32.0
3	2/3	267 ± 118*	13.5 ± 4.3*
Normal	NA	40.7 ± 7.3	2.8 ± 0.9

* $p < 0.03$ compared to normal, student's *t*-test

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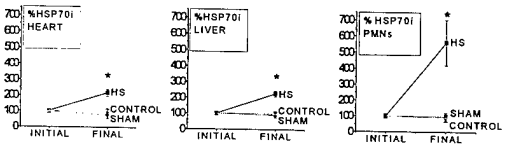
WHOLE LARGE-ANIMAL STRESS RESPONSE TO HEAT-SHOCK MA.Peñaranda*, M.C. Guisasola*, MJ. Sánchez*, F. Asensio*, A. Suarez*, I. Simón*, F.J.Villanueva*,P.García-Barreno, (Exper. Med.&Surg. Unit. Hospital General G. Marañón. Madrid, Spain).

Introduction. The pig is a large laboratory animal which shows many similarities to human. Cells from all the organisms respond to various stressors by the rapid expression of a set of highly conserved proteins known as heat-shock proteins (Hsps) or stress proteins. **Material and Methods.** The animals used in this study were handled in accordance with de European Communities Council guidelines for animal welfare. Male Maryland miniature swine were sedated and then anesthetized. After intubation, the pigs were mechanically ventilated. A V.cava cranealis temperature sensor was placed in all animals, and a restricted thoracotomy and laparotomy were performed in protocol and sham groups. Then, a stabilization period of 20-30 mins was allowed. Afterwards, the initial liver and transmural heart biopsies (protocol and sham groups), and peripheral venous blood (all groups) were obtained. Immediately, the protocol animals were submitted (2h) to an external heat source (blanket) to increase central blood temperature (from 38,5ºC to 42ºC). New samples of liver, heart, and blood were adquired after 3h of central hyperthermia in protocol animals, and after a similar period of time in the sham and control animals. Hsp70c and Hsp70i were studied in hepatocytes, myocytes, and central blood polymorpho-nuclear neutrophils (PMNs). PMNs isolation by gradient. Protein soluble fraction from homogenizate samples separated by one dimensional SDS-PAGE. HSP 70c and 70i detected by inmuno-blotting with specific mAbs and quantificated by Scilimage software. Statistical analysis: Wilconson test, non parametric ANOVA (Kruskas-Wallis test) and U-Mann-Whitney test. **Results and Discussion.** The liver, heart, and PMN constutive-Hsp70 (Hsp70c) behaviour does not change along the time in either of the three groups. It is important to signal that PMN-Hsp70c neither PMN-Hsp70i show any variation between the basal values and the initial point in the three groups. Moreover, the tissular-Hsp70i levels are stable along time in control and sham groups. However, the liver, heart, and PMN Hsp70i respond significantly to heat-shock. So, we think that all the data shown above back up the validity of the study since only the variable (heat-shock in our study) affects the response, and not the complex handling of the animal.



MEAN± SEM. * p <0,05 versus control, sham and initial level

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A NEW METHOD FOR MEASURING BLOODING LOSS WITH A GAMMA CAMERA, IN A CANINE MODEL OF UNCONTROLLED HEMORRHAGE. S Sinosaki*, EA Sallum*, FL Pereira*, FY Hondo*, R Abe*, RS Coimbra, PD Branco*, M Rocha e Silva. Research Division, Heart Institute-InCor, Univ. São Paulo, SP 05403, Brazil.

Introduction: Radioisotope scintilography and gamma camera imaging have been extensively employed. It is important for the assessment of renal function, cerebral flow, ventricular function, and for detection of bleeding sites ranging from orthopedic surgery to gastrointestinal bleeding. No reliable method for quantifying blood less has been described. This projects attempts to cover this point. *Methods and Results*: A portable gamma camera (Elscint Ltd, model Apex 209M) was positioned over the abdomen of dogs. To generate a standardized retroperitoneal hematoma (**RH**) a known volume of Tc^{99m} labeled donor blood was directly injected into the retroperitoneal cavity in 50-ml aliquots; gamma camera images were made at 5-min intervals. An additional image was obtained from a syringe containing 10 ml of labeled blood. Counts from a specific "region-of-interest" were made and compared to the syringe count. From injected volume, syringe volume, "region-of-interest" count and syringe count, a correction factor of 3.67 was established for this model. To generate a naturally occurring **RH** the common iliac arteries of dogs with Tc^{99m} labeled blood were punctured bilaterally. Serial abdominal images and a syringe image were likewise obtained. Pre-hematoma red cell volume was determined through Tc^{99m} dilution. The first image (before **RH**) was used as background count. Final circulating red cell volume was determined by subtracting **RH** volume from initial volume. In 5 other dogs, a similar natural RH was generated: initial and final circulating red cell volumes were determined from Tc^{99m} and Cr^{51} dilution, respectively. Results: Red cell loss was 31.2% in the first procedure, 32.8% in the second, with no significant differences between them ($p > 0.7$). Conclusions: This method of quantifying blood loss is potentially useful for the follow-up of experimental uncontrolled hemorrhage.

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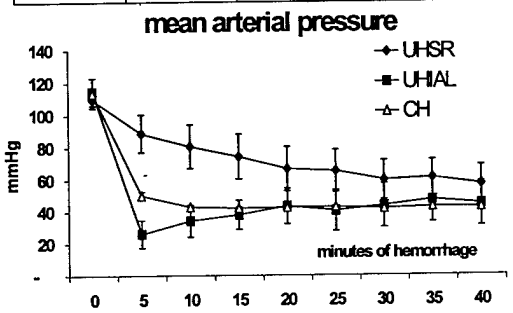
BLOOD LOSS AFTER CONTROLLED AND UNCONTROLLED HEMORRHAGE MODELS FOR PREHOSPITAL FLUID RESUSCITATION. EY Varicoda*, VS Bruscagin*, JLM Braz*, CB Murta*, S Rasslan*, M Rocha e Silva, LF Poli de Figueiredo. Research Division, Heart Institute-InCor, Univ. of São Paulo, SP 05403, Brazil

We compared blood loss and hemodynamic profiles between three distinct shock models: 1) uncontrolled hemorrhage from spleen rupture (UHSR) or 2) iliac artery lesion (UHIAL) and 3) controlled hemorrhage (CH), in which animals were bled to a mean arterial pressure of 40 mmHg in 5 min and maintained at these levels. Fifty-three spontaneously breathing anesthetized dogs (17±2 kg) were divided into 6 groups according to the model and moment of direct blood loss measurement: UHSR, UHIAL and CH after 20 and 40 min. Table1 displays blood loss in mL/kg.

	UHSR	UHIAL	CH
20 min	30±4	39±10	37±2
40 min	39±5	48±6	46±2



Splenic trauma induces a slower but progressive bleeding and shock; both iliac artery lesion and the classic controlled hemorrhage model induced abrupt bleeding and shock. Both uncontrolled hemorrhage models are useful to address controversies on fluid resuscitation after hemorrhage induced by blunt or penetrating abdominal injuries.

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TEMPERATURE DEPENDENCY OF BIDIRECTIONAL FLUX OF FLUORESCENTLY-LABELED DEXTRAN IN THE RAT INTESTINE SUBJECTED TO GRADED ISCHEMIA. S. Wattanasirichaigoon*, M. Menconi* and M. Fink. Department of Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215.

This study examined the effect of temperature and ischemia on permeation of fluorescently-labeled dextran (M.W. = 4 kDa; FD4) across rat intestinal mucosa. Permeability was evaluated ex vivo using an everted gut sac technique in both the mucosal-to-serosal (M→S) and serosal-to-mucosal (S→M) directions. At baseline (B), 30-min of ischemia (I-30) and 60-min of ischemia (I-60), intestinal segments were prepared and incubated at 37°C, 15°C and 4°C (n=4-7/group) for 30 min. M→S clearance (nl/min/cm^2) was calculated based on the accumulated amount of FD4 (S side) at 30 min and S→M clearance was calculated based on the slope of the accumulation rate of FD4 (M side). Both M→S and S→M fluxes increased with increasing temperature at B, I-30 and I-60. Ischemic gut (I-30 and I-60) had about a three-fold higher (M→S)/(S→M) flux ratio than that of normal gut ($p<0.001$). At 4°C, neither M→S nor S→M flux was different between B and I-30, but both M→S and S→M fluxes significantly increased at I-60, suggesting an increase in permeation via a passive mechanism. Increased bidirectional fluxes at 37°C were obtained in the I-30 and I-60 gut sacs when compared to B. We conclude that FD4 is actively transported across the intestinal mucosa in the S→M direction and that ischemic injury increases passive diffusion of the probe across the gut wall.

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THE RABBIT NEONATE AS A MODEL FOR METABOLIC STRESS A. Wei*, N. Ismail*, A. Elmishad*, L.O. Byerley*, and W.J. Chwals. Univ. Chicago, Chicago, IL 60637.

The goal of this project is to establish a neonatal model to study the metabolic changes elicited by a septic challenge. The metabolic stress response is poorly understood in the human neonate, and difficult to study. Adult New Zealand white rabbits (A) and 24 h neonatal rabbits (N) were injected with lipopolysaccharide (LPS) or saline (control). 24 h after the injection, serum CRP was significantly higher in the LPS groups with less of an increase in the LPS N compared to LPS A. At the same time, serum glucose was significantly lower in the LPS N than the control N. The LPS N tended to produce more glucose than the other 3 groups (primed, constant infusion of 6,6 d_2 glucose) and tended to clear more glucose ($p<0.064$). Serum glycerol and the rate at which glycerol was released from adipose tissue (primed, constant infusion of [$^5D_2$]glycerol) did not differ among the four groups.

mean ± SD	Control A	LPS A	Control N	LPS N
Wt (kg)	2.9±0.1*	2.8±0.2	0.07±0.01	0.06±0.02
CRP (mg/dL)	0.3±0.2	3.4±0.8**	0.2±0.2	1.9±0.7**
Glycerol (mM)	0.23±0.14	0.09±0.04	0.11±0.03	0.09±0.03
Glucose (mg/dl)	223±66	238±117	102±55	43±20***
Glucose R_a (µmol/min/kg)	48.8±29.3	63.0±27.3	81.8±16.9	94.9±37.2
Glycerol R_a (µmol/min/kg)	6.4±2.8	8.9±2.3	6.7±3.4	7.7±3.7
Glucose Clearance (ml/min/kg)	4.7±2.3	6.1±4.1	44.3±40.0	29.7±27.7

**P< .05, LPS A vs. LPS N; ***P< .007, Non LPS N vs. LPS N

Conclusions: Similar changes in carbohydrate and fat metabolism were observed in this model as previously described for stressed human neonates and adults. We can now examine mechanisms responsible for these differences. Establishing the *in vivo* cellular mechanisms controlling the neonatal stress response will lead to development of improved treatment strategies for the critically ill infant.

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PLATELETS RETAIN A NORMAL LIFE SPAN FOLLOWING SEQUESTRATION INDUCED BY ANTI-PROTEIN C ANTIBODY AND FACTOR XA-PCPS IN THE BABOON. R.Wolf*, A.Chang, G.Peer*, D.Carey, M.Lockhart*, F.Taylor Jr., Univ. of Okla. Health Sciences Center, Oklahoma City, OK 73104

Thrombin was generated *in vivo* by administration of a combination of Factor Xa (fXa)(36nM/kg) and phosphatidylcholine/phosphatidylserine (PCPS) vesicles (58µM/kg) to baboons (Papio sp.). Thrombin is a strong activator of platelets but at the dosages used platelet count remained stable unless the animals were pretreated with a monoclonal antibody which inhibits Protein C activation (HPC4)(1mg/kg), whereupon a sudden but transient drop in platelet count (13.9 ±0.13% n=3 of baseline concentration) was seen. The nadir was reached 2 to 5 minutes post fXa-PCPS infusion after which time the platelet count gradually returned to normal over the next 2-3 hours. By using an *in vitro* biotinylation protocol to label platelets it was determined that the platelets, rather than being consumed, were instead sequestered and later released back into circulation. The life span of these platelets was found to be indistinguishable (6.2 ±0.87 days n=3) from life spans in animals which did not receive fXa-PCPS and HPC4(6.2 ±0.83 days n=4). These studies show that thrombin induced platelet activation and transient sequestration does not decrease life span.

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ANTI-HUMAN PLATELET FACTOR 4-HEPARIN ANTIBODIES ARE CAPABLE OF ACTIVATING PRIMATE PLATELETS AS MEASURED BY ^{14}C-SEROTONIN RELEASE. S. Ahmad, W.P. Jeske*, J.J. Wood*, L.H. Yang*, K. Fu*, J.M. Walenga* and J. Fareed*. Cardiovasc. Inst. and Pathol. & Pharmacol. Depts., Loyola Univ. Chicago, Maywood, IL 60153.

Several previous studies from our laboratories have reported that platelets from primates *(Macaca mulatta)* behave in a similar fashion as the humans. Similarly, glycoprotein IIb/IIIa inhibitors (both synthetic and antibody-derived) are capable of antagonizing the activation processes in primate (monkey) platelets. It is now widely accepted that the patho-physiology of heparin-induced thrombocytopenia (HIT) is associated with the generation of anti-platelet factor 4-heparin (APF4-H) antibodies which are capable of activating platelets and endothelial cells through Fc receptors. To compare the effects of these APF4-H antibodies in human and primate platelets, ^{14}C-serotonin release method was employed. In the initial experiments, the ^{14}C-serotonin uptake and release induced by Triton X-100 and TRAP was found to be similar in human and primates ($p>0.09$, $n=18$). In the ^{14}C-serotonin release assay, heparin at 0.1U/ml concentration produced similar serotonin release in both the primates and human platelets ($76\pm7\%$ release, $p>0.1$, $n=18$). At concentrations $\geq$10 U/ml, heparin suppressed the ^{14}C-serotonin release in this assay. Immunoglobulins (IgGs) isolated from patients ($n=6$) with positive HIT responses were also found to exhibit the activation of human and primate platelets ($79\pm12\%$ release, $p>0.08$, $n=15$). Again, the platelet activation response was dependent on heparin concentrations. Unlike human platelets, the primate platelets exhibited a more consistent release response (15 out of 18 monkeys). In contrast, donors (normal healthy volunteers) showed a wide variations in the activation/release response. These results suggest that primate platelets are activatable by APF4-H antibodies presumably by having a homology in the Fc receptors and other physiologic mechanisms between the two species. This observation also supports the hypothesis that primates can be used to develop an animal model to study the pathogenesis of HIT (which oftern coexists with inflammatory or infectious conditions, such as autoimmune disorders, septicemia, malignancy, or trauma). Additional studies on primate Fc receptor characterization utilizing anti-Fc receptor antibodies and its interactions with the HIT antibodies are in progress at this time. The development of a sub-clinical model of primate HIT syndrome will be helpful in the understanding of the vascular responses (hemodynamic) in this disease. Furthermore, such a model can be used to study the comparative effects of various drugs in HIT syndrome.

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CHARACTERIZING RIGHT VENTRICULAR (RV) DYSFUNCTION DURING SEPSIS USING PRESSURE-VOLUME DIAGRAMS MC Chang, HM Russell*, EH Kincaid*, JW Meredith, Wake Forest Univ. Sch. of Med., Winston Salem, NC 27157

Introduction: Changes in RV afterload and contractility associated with sepsis are poorly described. Our goal was to use pressure-volume diagrams to describe RV dysfunction and track the effects of inotropes in a series of septic trauma patients. **Methods**: RV pressure-volume diagrams were constructed using data from the volumetric pulmonary artery catheter on admission, at onset of the Systemic Inflammatory Response Syndrome (SIRS), and with inotropic agents in a consecutive series of patients who developed post-injury SIRS and/or sepsis (SIRS+infection). Contractility was assessed via end-systolic elastance ($E_{es}RV$, mmHg/mL/m^2), and afterload via pulmonary artery input impedance (E_aPA, mmHg/mL/m^2) and pulmonary vascular resistance index (PVRI). RV ejection fraction (RVEF) was also measured. **Results**: 34 patients met criteria for SIRS. Inotropes were started to improve systemic perfusion 34 times in 31 patients (dobutamine n=21, dopamine n=10, or epi n=3).

Variable	Baseline	Onset	Treatment
$E_{es}RV$	0.66±0.23	0.50±0.23*	0.69±0.36**
E_aPA	0.87±0.25	0.99±0.36	0.95±0.33
PVRI	250±90	260±120	240±90
RVEF (%)	41±6.0	32±8.0*	39±8.0**
$p<0.05$, t-test w/ Bonferroni's vs Baseline* or Onset**			

$E_{es}RV$ decreased with the onset of sepsis. E_aRV and PVRI did not change. Inotropic agents improved $E_{es}RV$ to baseline values and did not affect RV afterload. There were no differences in the studied variables in SIRS vs septic patients. **Conclusions**: Decreased RV contractility characterizes post-traumatic SIRS and sepsis. SIRS did not affect right-sided afterload. Administration of inotropic agents improves RV contractility.

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THE ABDOMINAL COMPARTMENT SYNDROME – EVALUTION OF NORMAL INTRAABDOMINAL PRESSURE AFTER SURGERY

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I. Department of Surgery, University of Cologne, 50931 Cologne, Germany

Objective: During the last five years the concept of abdominal compartment syndrome has been identified as a cause for shock in trauma patients and after major abdominal surgery. Up to day there is no prospective data available on normal values of intraabdominal pressure. Aim of this study was to determine intraabdominal pressure and to compare it to those of patients, who had a higher risk to develop an abdominal compartment syndrome.

Design: Prospective study.

Patients and Methods: Intraabdominal pressure was measured indirectly using urinary bladder pressure in surgical ICU patients (1) after abdominal surgery (ventilated: n = 20, non-ventilated: n = 20) and (2) with non-abdominal surgery (n = 20). Intraabdominal pressure was also determined in two patients with an abdominal compartment syndrome. Significance was tested by ANOVA.

Results: No differences were detected in intraabdominal pressure between ventilated and non-ventilated patients who underwent abdominal surgery (12.5 mmHg ± 4,3 vs. 12.3 mmHg ± 4.7 mmHg; $p > 0.05$). The difference to patients who were operated due to non-abdominal reasons (9.3 mmHg ± 3.2mmHg) was not significant. The intraabdominal pressure of two patients with an abdominal compartment syndrome was 44 mmHg respectively 40 mmHg.

Conclusion: Intraabdominal pressure in patients after abdominal surgery can be assumed around 12 mmHg, for patients after non-abdominal surgery less than 9 mmHg. Patients with abdominal compartment syndrome can be identified by a three- to fourfold increase of intraabdominal pressure.

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RAPID DETECTION OF A DEFICIENT GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) GENETIC VARIANT IN TRAUMA PATIENTS. A.M. Liese*, M. Siddiqi and Z. Spolarics. Department of Anatomy, Cell Biology and Injury Sciences, UMDNJ- New Jersey Medical School and Graduate School of Biomedical Sciences, Newark, NJ 07103

Genetic predisposition to trauma-associated sepsis is an emerging field of interest. G6PD deficiency is the commonest human enzymopathy. The defect may cause chronic or acute hemolysis as a result of increased sensitivity of RBC to oxidative stress. Over 300 variants of the single copy gene (expressed in all cells) have been described. Currently, we investigate the effect of a common G6PD deficient variant on the clinical complications of injury. Here we report an easy and improved method for identifying a deficient G6PD A-202A/376G variant

(double mutation at nucleotides 202 and 376, present in 10-11% of African-American males). The technique employs allele-specific synthetic oligonucleotides and polymerase chain reaction. Four allele-specific sense primers were synthesized: two specific for the "normal" G6PD allele and two specific for the "deficient" allele. Allele-specificity of the sense primers relied solely on their 3¢-terminal nucleotides positioned complementary to nucleotides 202 and 376, respectively. Antisense primers were designed to obtain different sized PCR products. A separate primer pair was also designed (reactive with both deficient and non-deficient DNA) which served as a control for the PCR. PCR products were subjected to electrophoresis and genotype was determined by the presence or absence of amplified products. The assay identified normal and deficient G6PD alleles, and generated no false positives or negatives in 14 patients. Results were confirmed by restriction endonuclease analysis of the same DNA samples. The described method is rapid and reliable in routine laboratory screening of this common G6PD variant. The same approach may be used for the rapid identification of other G6PD point mutations in hospitals treating patient populations with high frequency of a particular variant. (Supp. by NIH GM55005)

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CYTOKINES AND DISTRIBUTION OF IMMUNE CELLS IN PATIENTS WITH SURGICAL HEPATO-BILIARY- PANCREATIC (HBP) DISEASE WITH AND WITHOUT JAUNDICE. M. Ljungdahl*, J. Österberg* and U Haglund. Uppsala University, Uppsala, Sweden

Jaundiced patients are considered to be at high risk to develop postoperative septic complications. To investigate if this could be related to defects in the splanchnic cellular immune cell distribution HPB patients with jaundice (**H+**, n=13, median age 58 (24-78) yrs) and without (**H-**, n014; 68 (47-89) yrs) were compared with controls operated for benign gastric disorders (**C**, n=10; 46 (22-64) yrs). The **H-** patients except 3 had previous (weeks) percutaneous or endoscopic draining procedures for jaundice. The bilirubin value of the **H+** patients was significantly elevated (92 ± 121 µmol/l). After informed consent blood samples were obtained preoperatively. At surgery two mesenteric lymph glands were excised from the ileo-cecal region. Plasma TNF-1α, Il-6 and Il-10 were determined using ELISA. Immune histology was performed using the monoclonal antibodies CD 4, CD 8 and CD 68, mainly staining T- killer, T-helper lymphocytes, and macrophages, respectively. WBC and CRP values were normal in all groups. TNF was 3.1 ± 0.8 (SD), 4.3 ± 0.7, and 3.4 ± 0.6 in the **C**, **H+** and **H-** patients, respectively. Corresponding values for Il-6 and Il-10 were 3.8 ± 0.8, 8.8 ± 1.9, 16.0 ± 9.1 and 12.7 ± 4.2, 11.1 ± 1.4 and 5.8 ± 0.7, respectively. **H-** patients had more CD 4 and CD 68 staining cells than **C** and **H+** patients ($p < 0.01$, Wilcoxon) but there was no significant difference between **H+** and **C**. In conclusion, surgical HBP disease is not associated with significant changes in plasma cytokines. Jaundice seems to inhibit the increase in T lymphocytes in mesenteric lymph glands otherwise seen in HPB patients.

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PLASMA ADRENOMEDULLIN IS INCREASED DURING AND AFTER MAJOR SURGERY. K. Okada, S. Fujioka*, and M. Karasawa* Dept. of Anesthesiology, Teikyo Univ, Tokyo, Japan.

Recent studies reported the relationships between Adrenomedullin (AM), a novel vasodilatory peptide, and cytokines and nitric oxide (NO). This study was performed to determine whether major surgery, which is accompanied with altered cytokines and NO production, affected plasma AM concentration. **Methods:** After institutional review board approval and informed consent, ASA I - II patients undergoing major upper abdominal surgery (MAS group, n = 16), and total hip arthroplasty (control group, n = 8) were studied. Arterial blood samples were obtained before anesthetic induction (PRE), 2 hour after surgical incision (2H), end of surgery (END), 1 post operative day (1POD), and 3POD. Plasma concentration of mature AM (active form of AM amidated at C-terminus) was measured using radioimmunoassay. Serum concentrations of interleukin (IL)-6, and nitrite/nitrate, a stable end product of NO, were also measured. **Results:** Plasma mature AM was increased during and after major upper abdominal surgery (table). There was a significant correlation between concentrations of plasma mature AM and serum IL-6 ($r = 0.541$, $p < 0.001$).

	PRE	2H	END	1POD	3POD
MAS group	1.4±0.3	1.9±0.5	4.0±0.5+*	4.3±0.6+*	3.0±0.7+
control group	0.8±0.1	0.9±0.1	1.1±0.2	1.1±0.2	1.9±0.5

mean ± SEM (fmol/ml), +P < 0.05 vs PRE, *P < 0.05 vs control group

Conclusion: The results suggest that major surgical stress might increase plasma AM concentration via cytokine induction.

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CYTOKINES AND CELLULAR IMMUNE RESPONSE IN PATIENTS WITH LOCAL AND GENERAL PERITONITIS. J. Österberg*, M. Ljungdahl* and U. Haglund. Uppsala University, Uppsala, Sweden

Peritonitis may cause changes in the splanchnic cellular immune system possibly mediated by cytokines. To further study the pathophysiologic events patients operated for appendicitis (**A**, n=14, median age 42 (20-74) yrs) or general peritonitis (**P**, n=11; 62 (36-84) yrs) were compared with controls (**C**, n=10; 46 (22-64) yrs). These latter patients had elective gastric surgery. After informed consent blood samples were obtained preoperatively. At surgery two mesenteric lymph glands were excised from the ileo-cecal region. Plasma TNF-1α, Il-6 and Il-10 were determined using ELISA. Immune histology was performed using the monoclonal antibodies CD 4, CD 8 and CD 68, mainly staining T killer lymphocytes, T helper lymphocytes, and macrophages, respectively. **A** and **P** patients had significantly elevated WBC (14.2 ± 3.2 (SD) and 13.1 ± 7.1,10^9 /l) and CRP values (95 ± 113 and 149 ± 145 mg/l, respectively; normal range <10). TNF was 3.1 ± 0.8 pg/ml, 6.5 ± 1.4, and 14.5 ± 5.5 in **C**, **A** and **P**, respectively. Corresponding figures for Il-6 was 3.8 ± 0.8,103 ± 41, and 373 ± 25, and for Il-10 12.7 ± 4.2, 45 ± 17, and 205 ± 69, respectively ($p<0.05$ when **P** was compared with **C**, for Il-6 **A** vs **C**; Wilcoxon). CD 4 and CD 68 staining cells were more frequent than CD 8 in all groups. **A** had more CD 8 positive cells than **C** ($p<0.01$) while the number of CD 4 and CD 68 staining cells were similar. There was no significant difference between **P** and **C** in any antibody staining cell. In con-clusion, general and local peritonitis caused significant changes in cytokine levels but only minor changes in the mesenteric lymph gland immune cell distribution.

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CONTINUOUS HEMODIAFILTRATION FOR ACUTE RENAL FAILURE DUE TO CARDIOGENIC SHOCK.
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Acute renal failure (ARF) is one of the most critical complications of cardiogenic shock. Hemodynamic instability induced by ARF makes it more difficult to apply hemodialysis (HD). In recent 2 years, we treated 6 patients with ARF due to cardiogenic shock (4; acute myocardial infarction, 2; acute aortic dissection) at first with continuous hemodiafiltration (CHDF). If hemodynamic instability recovered and doses of catecholamines were reduced, then we switched to intermittent HD. Results were shown in table 1. During this therapy, complications such as arrhythmia, hypotension or bleeding tendency were not observed. CHDF successfully controlled increase in serum urea nitrogen, creatinine and potassium levels, and improved hemodynamics. CHDF made it possible to switch to HD, which subsequently eliminated serum urea nitrogen and creatinine. In conclusion, CHDF was safely and effectively applied for the initial treatment of acute blood purification in patients with cardiogenic shock.

Table 1. Changes of clinical parameters after CHDF and followed HD. (n=6) (mean ± SD)

	Pre	Post-CHDF	Post-followed HD
UN	88.8±25.0	76.7±32.0 (n.s.)	49.5±12.8 (p=0.015)
Cre	6.43±2.51	5.13±1.32 (n.s.)	3.57±1.53 (p=0.041)
K^+	4.98±0.71	4.10±0.94 (n.s.)	3.92±0.69 (p=0.026)
Dop	7.28±2.46	2.30±2.09 (p=0.002)	0
BP(s)	121.3±9.69	134.33±11.3	133.5±10.8

Each data were compared to "Pre" group and analyzed by the Mann-Whitney test. Statistical significance was accepted at the 95% confidence level ($p < 0.05$). UN: urea nitrogen [mg/dl]; Cre: creatinin [mg/dl]; K^+: potassium [mEq/l]; Dop: dopamine [μg/kg/min]; BP(s): systoric blood pressure [mmHg].

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THE SYSTEMIC MEDIATOR ASSOCIATED RESPONSE TEST (SMART) PREDICTS END-ORGAN FAILURE IN SEPTIC SHOCK

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Previously SMART models from a purely septic shock database were validated. This study tested whether or not SMART predictions built on a comprehensive severe sepsis/septic shock cohort could be validated prospectively in septic shock patients.

Methods: Demographics and baseline physiologic data, standard laboratory tests, and ELISA levels of IL-6, IL-8, and GCSF from 200 septic patients were integrated into stepwise multiple logistic regression models that predicted shock, mechanical ventilation (VENT) and lung (ARDS), coagulation (DIC), hepatobiliary (HEPBIL), and renal failure. Prospective validation used baseline data from a separate septic shock cohort (n=48).

Results: Receiving operating characteristics area under the curve (ROC AUC) of predicted versus observed plots at 1, 3, and 7 days after baseline included:

Day	ARDS	DIC	HEPBIL	RENAL	Vent	Shock
1	0.711	0.906	0.880	0.892	0.858	0.591
3	0.682	0.791	0.668	0.849	0.753	0.537
7	0.756	____	0.813	0.837	0.869	0.653

Conclusion: The comprehensive SMART sepsis database predicts organ failure in individual septic shock patients.

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INCREASED TENDENCY OF SEPTIC EPISODES AND ALTERED MONOCYTE FUNCTIONS IN GLUCOSE-6-P-DEHYDROGENASE (G6PD) DEFICIENT TRAUMA PATIENTS.
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Genetic predisposition to septic complications in trauma is an emerging field of interest. The aim of our ongoing investigations is to determine if the common, type A$^-$ G6PD deficiency alters the clinical course of injury, and if the defect is manifested in altered leukocyte functions. Clinical data are collected prospectively, and oxidative burst and CD11b (integrin α-chain) expression in neutrophils and monocytes are tested on day2 and day5 post-injury by flow cytometry. Screening 467 patients with Injury Severity Score (ISS) ≥ 9 identified 39 deficiencies with less than 20% of residual G6PD activity in RBC. The mutations were also identified by allele-specific restriction endo-nuclease digestion in all patients. No adverse clinical effects were found comparing deficient (n=32) and non-deficient (n=33) patients with ISS ≥ 9<13. However, in severely injured patients (deficient: ISS=29±3.2, n=5; normal: ISS=26±2.3, n=10), the deficiency is accompanied by an increased tendency of septic complications. This is reflected in significantly longer (~3x) hospital stay, antibiotic therapy, SIRS days in deficient patients compared to controls. Bacteremia was found in 3 of 5 deficient versus 1 of 10 non-deficient patients. At day5 post-injury, phorbol-stimulated cell-associated peroxide content was significantly greater in deficient monocytes than in non-deficient cells, whereas basal or phorbol-stimulated CD11b expression were not different in deficient and non-deficient cells. This suggests that elimination of reactive oxygen species is compromised in deficient monocytes. Although the number of investigations is small, the data suggest increased septic complications and altered monocyte functions after severe injury in G6PD deficiency. (Supported by NIH, NIGMS, GM55005).

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INTRAVASCULAR VOLUME EXPANSION DURING THERAPEUTIC MODERATE HYPOTHERMIA FOR BRAIN-INJURED PATIENTS: A PRELIMINARY REPORT.
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Hemodynamic depression during moderate hypothermia may worsen the cerebral circulation after brain injury. In

this study, we examined retrospectively the effects of intravascular volume expansion on hemodynamic changes, intracranial pressure (ICP) and internal jugular oxygen saturation (SJO2) in seven brain-injured patients, who were selected because of their elevated ICPs even after inducing hypothermia. All patients were ventilated and underwent hypothermia of 32-33 °C induced by surface cooling using midazolam, buprenorphine and vecuronium. After the hypothermic period (dividing the period into the initial, middle, and late phases), patients were gradually rewarmed at a rate of approximately 1°C per day. Mean blood pressure (MBP), central venous pressure (CVP), cerebral perfusion pressure (CPP), cardiac output (CO), ICP and SJO2 were measured. Despite a large amount of infusion ranging from approximately 4000 to 5000 ml/day, ICP decreased from the middle phase as compared to the initial phase of the therapy. After such volume expansion, levels of CVP (ranging from 10±1 to 11±2 mmHg) was found during the hypothermic period, in association with CO (5.2±0.1 to 5.3±0.2 L/min), to be similar to those of normothermia. Sustained CPP was accompanied by reduced ICP, increased SJO2 and augmented CO. These results suggest that even a large amount of infusion to the brain-injured patients decreases ICP and improves CO during moderate hypothermia, which may be of beneficial to the cerebral circulation and metabolism in the patients. The current study warrants furhter future studies to test such an hypothesis.

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CECAL LIGATION AND DOUBLE PUNCTURE (2CLP) INDUCES ACUTE LUNG INJURY (ALI) / ADULT RESPIRATORY DISTRESS SYNDROME (ARDS) IN RATS.

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Most animal models for ALI and ARDS poorly mirror the clinical disease. We hypothesize that histologic and functional changes characteristic of ALI and ARDS will be found in the lungs of rats after 2CLP. All studies conformed to NIH guidelines. Sepsis was induced in rats via 2CLP with an 18 gauge needle,. Sham operated animals served as controls. Lung tissue was collected at 0, 24, 48, 72 hours in both groups. Light-microscopy was performed on H&E stained fixed sections. Wet:dry weight ratio, protein content in the bronchoalveolar lavage (BAL) fluid and myeloperoxidase (MPO) were determined. H&E stained lung sections in septic animals showed thickened alveolar septa and intraalveolar exudate with hyaline membranes, erythrocytes and neutrophils. There was a spectrum of disease ranging from mild injury to severe atelectasis and honeycombing. 2CLP lungs also showed an increase in wet:dry weight ratio, an increase in BAL proteins and MPO content. We conclude that cecal ligation and double puncture induces histological and functional changes characteristic of ALI and ARDS, including regional variability in the severity of disease. These findings provide a clinically relevant animal model for studying a variety of interventions for these disorders.

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THE RATIO OF ELR- TO ELR+ CXC CHEMOKINES AFFECTS NEUTROPHIL-DEPENDENT LUNG AND LIVER INJURY FOLLOWING HEPATIC ISCHEMIA/REPERFUSION.

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Lobar hepatic ischemia/reperfusion (I/R) results in a neutrophil-dependent lung and liver injury. The process of neutrophil recruitment and activation in this injury is at least partially dependent on the presence of the ELR+ CXC chemokines. Investigations have shown that ELR- CXC chemokines can inhibit ELR+ CXC chemokine neutrophil recruitment and activation in vitro. In order to begin to assess the role of the balance between these two types of molecules in vivo in neutrophil recruitment and activation in the setting of hepatic I/R, we used our rat model of lobar hepatic I/R and pre-treated animals with pharmacologic doses of gamma-interferon (γ-IFN). γ-IFN is known to upregulate two of the ELR- CXC chemokines, specifically γ-IFN-inducible protein (IP-10) and monokine-induced by γ-IFN (MIG). Following lobar hepatic I/R or sham laparotomy, animals were sacrificed at 1, 6, 12, and 24 hours. Hepatic and pulmonary levels of the ELR- CXC chemokines, IP-10 and MIG, and the ELR+ CXC chemokines, macrophage inflammatory protein-2 (MIP-2) and rat cytokine-induced neutrophil chemoattractant (KC), were determined by ELISA. Pulmonary neutrophil influx was quantitated by pulmonary myeloperoxidase (MPO) levels, hepatic neutrophil influx was quantitated with hepatic tissue morphometrics, lung injury was estimated by extravasation of Evans Blue dye as a measure of pulmonary capillary injury, and liver injury was estimated with serum ALT levels. The ratio of ELR- to ELR+ CXC chemokines in both the liver and lung was increased in response to γ-IFN, indicating an increase in the levels of the ELR- molecules as compared to the ELR+ molecules (Liver at 12°: I/R, ischemic lobe=0.48±0.07 vs I/R+IFN, ischemic lobe=1.93±0.2,$p<0.005$, Lung: I/R=3.32±0.44 vs I/R+IFN=5.18±0.74, $p<0.05$). In response to γ-IFN, there was a significant decrease in liver injury as measured by serum ALT (at 6°: I/R=26,690±9266 vs I/R+IFN=8878±2591, $p<0.05$) and lung injury as assessed by extravasation of Evans Blue dye (at 12°: I/R=0.61±0.08 vs I/R+IFN=0.32±0.04, $p<0.005$). Although there was a decrease in injury in both of these tissues, there was not a significant decrease in the presence of neutrophils in either tissue. This suggests that this alteration in the balance of ELR- to ELR+ CXC chemokines results in a decrease in neutrophil activation, but not neutrophil recruitment.

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INTESTINAL ISCHEMIA/REPERFUSION (II/R) INCREASES SERUM THROMBOSPONDIN-1 (TSP-1).

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TSP-1, an important mediator of inflammation, induces neutrophil (PMNs) adhesion, spreading and motility. This study examines the hypothesis that II/R injury increases serum TSP-1. Anesthetized male Sprague-Dawley rats (350gm) with arterial pressure (BP) monitoring underwent SMA occlusion for 60 minutes followed by 60 minutes of reperfusion (60/60) or sham (120SM) plus saline carrier (NS) or pentoxifylline (PTX, 50mg/kg). Plasma was analyzed for TSP-1, IL-1 and TNF_α levels by ELISA and prekallikrien (PK) by chromagenic assay at 55 min occlusion (55minI) and 60 min following reperfusion (60minR). Data reported as mmHg-BP, pg/ml-TSP-1 and % of baseline (BL) for PK (mean ± SEM, N≥6, analyzed by ANOVA ,*-$p<.05$ vs SM, **$p<.05$ vs 60/60).

	120SM	120SM+PTX	60/60	60/60+PTX
BP 55minI	93.0± 4	99 ±16	101±10	99 ±19
BP 60minR	62.0±10	52 ± 9	61±11	47 ± 6
TSP-1/BL	0	0	0	0
TSP-1/55minI	7.2±0.3	1.5±0.5	13±1.8	1.1± 0.8
TSP-1/60minR	3.4±0.1	4.9±1.5	9±0.6*	5.3±0.2
PK/55minI	94.0±1.5	92.0±1.4	83±1.2	88.9±1.7
PK/60minR	113.2±2.0	87.1±1.5*	120±2.9	76.3±1.6**

The 60/60 time of II/R did not alter plasma levels of IL-1 or TNF_α. II/R for the 60/60 time periods increased plasma TSP-1 concentration but did not alter serum concentrations of IL-1 or TNFα. PTX treatment did not reverse elevated TSP-1 levels following II/R but did decrease PK levels and blood pressure. These data suggest that TSP-1 is an indicator of the evolving systemic inflammation stimulated by II/R injury and may be one of the factors contributing to the systemic activation of neutrophils following II/R in the rat. These data also suggest that PTX activates the contact system of blood coagulation with release of bradykinin and secondary decreased arterial blood pressure.

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CYTOKINE CHANGES IN PLASMA AND PERITONEAL LAVAGE FLUID IN PATIENTS WITH SEVERE ACUTE PANCREATITIS. S. Kawaguchi, M. Aibiki, S.Ogura, M.Nakano, T.Nishiyama, K.Seki, Y. Kinoshita. Dept. of Anesthesiol, and Emerg. Med., Kagawa Med. Univ. 1750-1, Ikenobe, Miki, Kita, Kagawa, 761-0793, Japan.

Cytokines, such as interleukin (IL)-6 and IL-8, may be key mediators in the development of subsequent organ failure induced by severe acute pancreatitis. We measured both plasma and peritoneal lavage fluid (PLF) levels of IL-6 and IL-8 in seven patients diagnosed with the disease, by Japanese criteria. Peritoneal lavage was done for more than seven days for the purpose of removing necrotic tissue and mediators released into the peritoneal cavity. Instilled fluid for the lavage consisted of bicarbonate Ringer solution (Sublood™) and ulinastatin (UTI: a potent protease inhibitor, Mochida Pharm. Tokyo, Japan). Continuous injection of UTI with an antibiotic (imipenam) to the celiac artery was also performed in all patients. The take-up ratio of PLF instilled was approximately 85-90%, so, cytokine levels in PLF were standardized according to this ratio. Statistical analyses were done by ANOVA with Scheffe's F-test ($p<0.05$). This study was approved by the Ethics Committee. Extremely high levels of IL-6 and IL-8 in PLF declined sharply after the lavage, but both cytokine levels in plasma decreased more gradually over more than five days. Initial differences between plasma and PLF were lessened remarkably. These changes were associated with a rapid drop in lactate dehydrogenase (LDH), an indicator of tissue damage, in PLF. All patients treated with these procedures recovered completely.The results suggest that peritoneal lavage presented here, which is superior in outcome to any reported previously, could be a choice of treatment for patients with severe pancreatitis, and also indicate a need for further studies to determine whether early reductions of the cytokine differences between plasma and the peritoneal cavity is beneficial in the treatment of acute severe pancreatitis.

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HEAT SHOCK PROTEIN 72 SUPPRESSES MACROPHAGE TNFα PRODUCTION BY INHIBITION OF NFκB INTRANUCLEAR TRANSLOCATION

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Heat stress suppresses tumor necrosis factor-α (TNFα) production in mononuclear cells through unknown mechanisms. While heat shock protein 72 (Hsp72) has been implicated as a mediator, the effect of this inducible stress protein per se on macrophage TNF-α production is unclear. NFκB intranuclear translocation following IκBα degradation is a critical step in the activation of TNFα gene transcription. The influence of Hsp72 on IκBα and NFκB remains to be determined. We **hypothesized** that Hsp72 suppresses macrophage TNFα production by regulating NFκB subcellular distribution. The **purpose** of this study was to determine the role of Hsp72 in the regulation of macrophage TNFα production and its effect on NFκB translocation. **Methods:** Rat peritoneal macrophages (Mϕ) were isolated and subjected to heat stress (43 °C for 30 min. followed by a 4 h recovery) or treatment with liposomal recombinant Hsp72 . The expression of Hsp72 was examined after heat stress by immunoblotting and immunostaining. Following stimulation with LPS (200 ng/ml), IκBα degradation was examined by immunoblotting, NFκB subcellular distribution by immunofluorescent staining and total TNFα production by ELISA. **Results:** Heat stress induced Hsp72 in both the cytoplasm and the nucleus. Prior heat stress prevented LPS-induced IκBα degradation and NFκB intranuclear translocation and reduced TNFα production. The effects of heat stress on NFκB subcellular distribution and TNFα production were mimicked by liposomally introduced Hsp72. **Conclusions:** This study demonstrates that Hsp72 suppresses Mϕ TNFα production by regulating NFκB subcellular distribution. These findings suggest that Hsp72 is a critical mediator of heat stress induced resistance to inflammation. Furthermore, liposomal delivery of Hsp72 may offer a clinically feasible anti-inflammatory therapy.

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FETUIN, A UBIQUITOUS SERUM PROTEIN, INHIBITS EDEMA FORMATION AND TNF. M. Ombrellino*, H. Wang*, J. Che*, J.Vishnubhakat*, L. Borovikova*, M. Zhang*, L. Scher*, S. Friedman*, KJ. Tracey, Department of Surgery, Division of Vascular Surgery, North Shore University Hospital and The Picower Institute for Medical Research, Manhasset, NY 11030

The overproduction of proinflammatory mediators by activated macrophages mediates acute inflammation. We recently reported that fetuin, a negative acute-phase glycoprotein, is an anti-inflammatory mediator because it opsonizes cationic macrophage deactivating molecules. Asialofetuin lacks the sialic acid residues and does not block TNF synthesis. As the role of fetuin in acute inflammation is unknown, here we utilized the standard carrageenan pedal edema model. Male Lewis rats were treated with bovine fetuin (500 mg/kg) intraperitoneally (i.p.), asialofetuin (500 mg/kg) i.p. or 0.9% NaCl (2 ml) i.p. One hour later, 0.1 ml of 1% λ-carrageenan and 0.1 ml of 0.9% NaCl were injected to the left and right paw respectively and the difference (Δ) between paw swelling was measured at three hours. Paw edema fluid was measured for TNF by ELISA.

*p < 0.05 vs. control N.D: Not Determined

Group	Δ Paw Edema (mm)	Total TNF (pg/paw)
Control	2.66 ± 0.12	817.37 ± 177.88
Asialofetuin	2.30 ± 0.10	N.D.
Fetuin	1.51 ± 0.17 *	454.92 ± 178.86 *

All data is presented as mean ± SEM (n=6)

Fetuin significantly attenuates the development of acute edema and TNF production. We conclude that exogenously administered fetuin can suppress the development of acute inflammation.

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ANTI-EPCR ANTIBODY EXACERBATES THE HEMOSTATIC AND INFLAMMATORY RESPONSES AND ENDOTHELIAL INJURY RESPONSE TO SUBLETHAL *E. COLI*. FB Taylor, Jr, DK Kurosawa, S Kurosawa, G Ferrell, ACK Chang, Z Laszik, J Mollica, S Kosanke, G Peer,*CT Esmon. Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

Previous studies of the baboon model of *E. coli* sepsis have shown the importance of protein C in the regulation of both the hemostatic and inflammatory responses to *E. coli* (*JCI*, 79:918, 1987). The discovery of endothelial C receptor (EPCR) and the definition of its role as an amplifier of the activation of protein C by the thrombin/thrombomodulin complex raised the question of its importance in the regulation of the baboon response to *E. coli*.

Three groups of baboons were infused with sublethal concentrations of *E. coli* organisms. The first two groups received 5mg/kg of blocking (mAb #1494, N=4) and non-blocking (mAb #1510, N=3) monoclonal antibodies to EPCR while the third group received buffered saline, (N=7). Vital signs were observed and blood was collected at T-0, +1, +2, +4, and 6 to 8 hours for CPC, and clinical chemistries, ELISA assays of inflammatory and hemostatic components, and histopathologic studies of tissues from the non-survivors of the blocking antibody group.

Those animals receiving blocking antibody to EPCR plus sublethal *E. coli* survived from 7 to 54 hours. They exhibited a fulminant (DIC), an elevated creatinine, SGPT, microvascular thrombosis with hemorrhage of both the adrenal and renal cortex, and an intense influx of neutrophils into the adrenal, renal and hepatic microvasculature. Those animals receiving non-blocking antibody to EPCR plus sublethal *E. coli* were permanent survivors, the laboratory marker responses of which were significantly less severe than those of the blocking antibody group.

We concluded that the binding and accelerated activation of protein C to the EPCR receptor is an additional critical step in the regulation of the response to *E. coli* by components of the protein C network.

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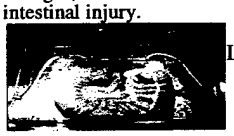
USE OF AN EXTRA-CORPOREAL CIRCUIT TO EVALUATE ISCHEMIA AND REPERFUSION IN THE EQUINE LARGE COLON L.VanHoogmoed*, N.Vatistas*, J. Snyder*, J Nieto*Univ. Calif. Davis School of Vet Med, Davis CA 95616.

A novel extra-corporeal circuit was developed to simulate ischemia and reperfusion injury in the large colon to determine the efficacy of this circuit to maintain a segment of large colon for 3.5 hours, evaluate low arterial flow on the histologic and hemodynamic variables, and evaluate the role of nitric oxide on contractile activity of the injured tissue. Data recorded included flow, pressure, temperature, perfusate, weight, and hemodynamic variables at 30 minute intervals for 3.5 hours with histologic evaluation at the study conclusion. Low flow for 40 minutes was followed by reperfusion for 2 hours. Circular muscle strips were cut from pre and post circuit tissue, mounted in a mechanical testing system and electrical field stimulation (EFS) performed to evaluate nitric oxide. The effects of a nitric oxide synthase inhibitor were evaluated on the inhibitory response generated by EFS. No significant difference in superficial and glandular epithelial loss, or interstitial to crypt (I:C) ratio occurred after 3.5 hours. After low flow and reperfusion, there was a significant difference in %superficial epithelial loss, glandular epithelial loss, hemorrhage and edema scores, and I:C ratio, perfusate volume, $PaCO_2$,arterial and venous bicarbonate concentrations, PaO_2, tissue weight, and arterial and venous base deficits. No significant differences occured in electrolyte or glucose levels. No significant difference was detected during EFS in inhibitory activity of the low flow group relative to the control tissue in the absence or presence of L-NAME. The circuit maintained the tissue for extended periods of time with minimal histologic changes, and can be manipulated to simulate the intestinal injury.



Large colon in isolated circuit

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EFFECT OF SPHINGOMYELINASE AND CONCURRENT LPS TREATMENT ON PGE_2 PRODUCTION BY HUMAN MICROVESSEL-DERIVED ENDOTHELIAL CELLS. John T. Flynn. Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

There is interest in the role of the sphingomyelinase /ceramide cycle as a mediator of specific signal transduction pathways. These include signaling by TNFα, interleukins, vitamin D, interferon γ and LPS. The present experiment was undertaken to compare the effect of sphingomyelinase (SMase) treatment with that of LPS upon PGE_2 production by a human adipose-derived endothelial cell line (HADMEC-5). Cells were grown *in vitro* and treated with LPS (10 ng/ml), vehicle or sphingomyelinase for 20 hours. PGE_2 production was evaluated by RIA. Cells treated with 0, 0.006, 0.06, 0.6 or 1.2 units/ml of neutral SMase demonstrated PGE_2 values of 0.6±0.1, 1.3*±0.3, 3.1*±0.6, 10.3*±1.9 and 11.1*±1.8 ng $PGE_2/10^5$ cells/20 hours respectively (mean±SE; n=12; *=$p<0.05$ vs 0 conc.). Treatment of cells with 10 ng/ml LPS for 20 hrs. resulted in a final production rate of 14.0±3.5 ng $PGE_2/10^5$ cells/20 hrs. Concurrent treatment of cells with both LPS and SMase (0.006, 0.06, 0.6 and 1.2 units/ml) resulted in PGE_2 production of 13.2±3.5, 22.6±5.4, 18.2±1.5 and 28.3±3.0 ng $PGE_2/10^5$ cells/20 hours respectively. Thus, there was an additive effect of LPS and SMase at the higher SMase concentrations. These data demonstrate that neutral sphingomyelinase is capable of directly initiating eicosanoid formation by microvessel-derived endothelial cells *in vitro* to an extent similar to that of a high dose of lipopolysaccharide (10 ng/ml). Furthermore, the effect of SMase upon PGE_2 production appears to be additive to the effect of LPS. This work was supported by grant 28023 from the NIGMS, NIH.

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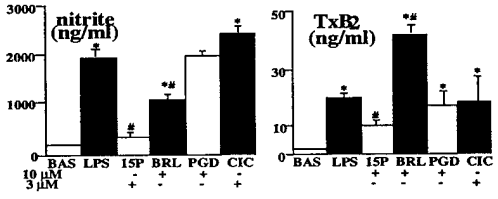
INHIBITION OF MACROPHAGE MEDIATOR PRODUCTION BY THE PROSTAGLANDIN J_2 (PGJ_2) METABOLITE: 15-DEOXY-$\Delta^{12,14}$ PGJ_2 K Guyton, R Bond*, S Ashton*, G Tempel*, C Wise*, PV Halushka*, and JA Cook*; Depts of Micro and Imm, Phys, Pharm and Med; MUSC, Charleston SC; Dept of Phys, U of SC, Columbia, SC

Recent studies suggest that the newly characterized PGJ_2 and its metabolites, including 15-deoxy-$\Delta^{12,14}$ PGJ_2 (15P) possess antiinflammatory properties. Unlike other eicosanoids, PGJ_2 and its metabolites, which derive from PGD_2 have no known plasma membrane receptor. It is postulated that 15P elicits its effects by activation of the nuclear receptor, peroxisome-proliferator activated receptor gamma (PPARγ). We hypothesized that 15P modulates macrophage (MØ) mediator release through PPARγ activation. The effects of 15P were compared to a specific PPARγ agonist, BRL 49653 (BRL), and to the eicosanoids, PGD_2 and cicaprost (CIC; a PGI_2 analogue) which activate plasma membrane receptors distinct from PPARγ. Rat peritoneal MØ were stimulated *in vitro* for 24 hours with endotoxin (LPS; 10 μg/ml) alone or with each eicosanoid. Supernatants assayed for LPS-induced nitric oxide (NO), determined by nitrite, and thromboxane B_2 (TxB_2) production, determined by RIA. (data below).



*Data are mean ± s.e.m for n = 3-4 * $p < 0.05$ vs control basal, # $p < 0.05$, compound + LPS vs LPS alone .*

15P and BRL both inhibited LPS-induced nitrite production. 15P also inhibited LPS-induced TxB_2 production. In contrast, BRL augmented TxB_2 production. Neither PGD_2 or CIC affected LPS stimulated nitrite or TxB_2 production. The results raise the possibility that 15P may produce effects through both PPARγ dependent and independent pathways. Supported by NIH GM27673.

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EFFECT OF PROSTAGLANDIN SYNTHESIS INHIBITORS ON NEUTROPHIL Ca^{2+} SIGNALING IN SEPSIS. A. Kohn*, M. A. Choudhry, and M. M. Sayeed. Department of Physiology and Burn & Shock Trauma Institute, Loyola University Chicago, Maywood, Illinois 60153.

Neutrophils play an important role in remote tissue damage after trauma and burn injuries, and some studies have found that prostaglandins can inhibit the activated neutrophils. Recent studies in our laboratory have shown that prostaglandins attenuate sustained Ca^{2+} influx. The present study investigated whether prostaglandins modulate Ca^{2+} signaling in neutrophils after sepsis by blocking prostaglandin synthesis. To induce sepsis, Sprague Dawley rats were implanted, intra-abdominally, with fecal pellets containing *Escherichia coli* (100 CFU) and *Bacteroides fragilis* (10^4 CFU). Sham rats were implanted with sterile pellets. 24 hours later, a group of rats were injected with prostaglandin synthesis inhibitors (COX I or II inhibitors, 10 μg/kg), or vehicle (DMSO) and sacrificed two days after implantation. Neutrophil cytosolic $[Ca^{2+}]$ was determined before and after stimulation with fmlp (1μM). Basal and fmlp (1μM) mediated elevation in $[Ca^{2+}]_i$ (Δvalues) were:

	Control	Sterile	Sepsis	Sep+COX I Inhibitor	Sep+COX II Inhibitor
Basal	89±21*	164±4	202±16	260±24	333±77
Δvalue	112±0.5	118±14	94±3	285±38	229±26

*Values (±SD) in nmol/liter.

The treatment of septic animals with prostaglandin inhibitors (COX I or II inhibitors) apparently caused a further increase in basal $[Ca^{2+}]_i$ along with a significant elevation in Δvalues in activated neutrophils. Further studies are being conducted to elucidate these findings. (Supported by NIH grants GM 53235 and GM 568501).

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EFFECT OF SELECT CYCLOOXYGENASE (COX)-1 AND COX-2 INHIBITORS ON PROSTAGLANDIN PRODUCTION AND T-CELL PROLIFERATION IN SEPSIS. S Lanza-Jacoby, S Miller, JT Flynn. Thomas Jefferson Univ., Phila., PA 19107.

The immunosuppression of T-cell function during sepsis is thought to be mediated by an increase in the production of prostaglandin (PG) E2 by splenic macrophages. The purpose of this study is to determine whether selectively blocking cyclooxygenase (COX)-1 or COX-2 will inhibit production of PGE2 by splenic macrophage and whether this decrease in production will restore T-cell proliferation in sepsis. PG production and T-cell proliferation were evaluated in control and septic rats after in vivo and in vitro treatment with COX-1 (SC560) and COX-2 (NS398, SC236) inhibitors. Sepsis was induced in male Sprague-Dawley rats, 250-275g, by cecal ligation and puncture (CLP). Rats were pretreated 30 min prior to CLP with i.p. injection of ibuprofen, SC560, SC236, NS398, and SC560 +SC236. PGE2 concentration was measured by RIA in the supernatant harvested from Con A-stimulated splenocytes, +/- inhibitors, after a 24 hr culture. T-cell proliferation was determined by measuring ^{3}H-thymidine uptake after 72 hrs stimulation with Con A. Con A-stimulated PGE2 production increased over 5-fold in splenocytes from 18 hr CLP rats compared with control rats. Pretreatment with SC236, NS398, SC236+SC560, and ibuprofen attenuated the rise in PGE2 production in Con-A-stimulated splenocytes from CLP rats. Sepsis decreased T-cell proliferation by 53% in comparison to sham control rats. Ibuprofen and SC236+SC560 restored T-cells proliferation in CLP rats while SC236 and NS398 partially restored proliferation. These findings suggest that COX-2 contributes to the rise in PGE2 production by splenic macrophages, and that inhibiting COX-2 will improve T-cell proliferation during sepsis.

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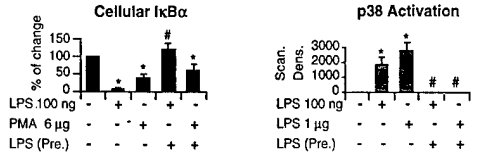
ENDOTOXIN DESENSITIZATION INDUCES RAPID DOWNREGULATION OF CD14 RECEPTOR COUPLED EARLY SIGNAL TRANSDUCTION EVENTS. M. Ferlito, F. Squadrito*, P.V. Halushka* and J.A. Cook* Depts. of Phys., Pharm., and Med., Med. Univ. of S.C., Charleston, S.C.

Prior exposure to endotoxin, a bacterial lipopolysaccharide (LPS), induces desensitization to LPS restimulation *in vivo* or *in vitro* in LPS responsive cells. Since desensitization occurs without a decrease in CD14 expression, post-receptor alterations such as impaired NF-κB activation have been implicated. We hypothesized that signaling changes induced by LPS desensitization are a consequence of decreased degradation of the NF-κB inhibitor peptide, IκBα, and impaired activation of a mitogen activated kinase (p38). Degradation of IκBα and activated p38, (measured by anti-active p38α/β antibody), were quantitated in Chinese Hamster Ovary cells (CHO) transfected with human CD14 receptor (CHO-CD14) and vector only (CHO) cells. LPS (10 ng/ml-1 μg/ml) stimulated rapid (30 min.) degradation of IκBα and activated p38 in CHO-CD14 cells but not in vector only cells. CHO-CD14 cells were desensitized by pretreatment (Pre.) with LPS (100 ng/ml) for 2.5 hrs or pretreated with LPS vehicle, washed, and restimulated with LPS (100ng or 1 μg/ml) or phorbol myristate acetate (PMA) 6 μg/ml for 30 min. (Data below).



Scanning densimetric values expressed as mean ± sem (n=3-8)
**p<0.05 vs basal: #p<0.05 LPS Pre.vs LPS.*

These studies demonstrate that LPS induction of IκBα degradation and p38 activation are CD14 coupled signaling events. LPS desensitized CHO-CD14 cells become rapidly (2.5 hrs) refractory to these LPS stimulated signaling events. Nonspecific activation of LPS desensitized cells with PMA was unimpaired. These findings support the hypothesis that LPS desensitization induces rapid changes in CD14 coupled signal transduction by preventing IκBα depletion, thus inhibiting NF-κB activation, and by inhibiting activation of p38. Supported by NIH GM27673.

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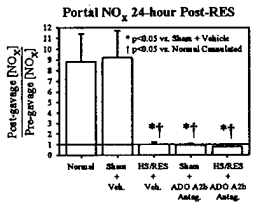
GLUCOSE-INDUCED, ADENOSINE-MEDIATED PORTAL NITRIC OXIDE PRODUCTION IS IMPAIRED AFTER RESUSCITATION FROM HEMORRHAGE. PJ Matheson, MA Wilson, CA Conn, MB Carter, RN Garrison. University of Louisville and Louisville VAMC, Louisville, KY.

INTRODUCTION: Na^+-linked D-glucose absorption increases intestinal blood flow by adenosine (ADO) release, which stimulates nitric oxide (NO) production via ADO A2b receptor activation. Previous studies have shown: 1) early protection against intestinal microvascular vasoconstriction after hemorrhagic shock (HS) by mucosal glucose exposure, and 2) early impairment of intestinal NO synthase activity following resuscitation (RES) from HS. Therefore, we hypothesized that

glucose-induced, adenosine-mediated NO production might be chronically reduced following HS/RES. METHODS: Anesthetized, male Sprague-Dawley rats (n=32) were cannulated, volume-HS (15mL/kg, femoral vein) and RES (shed blood + 2 vol normal saline). Sham animals underwent surgical manipulation but not HS/RES. At 24 hours post-RES, animals received an ADO A2b receptor antagonist (alloxazine, 150 μg/kg/25 min, IV) or vehicle prior to D-glucose (1.5cc, 5%) gavage. Portal serum samples (BL and 20-min post-gavage) were obtained and assayed for NO metabolites (NO_x) by fluorescent-modified Greiss assay. RESULTS: All graphical data are expressed as post-gavage $[NO_x]$/BL $[NO_x]$ ± SEM. D-Glucose gavage increased portal vein NO_x in normals and shams which was prevented by ADO A2b blockade. BL NO_x levels at 24-hr post-RES were elevated compared to Normal and Sham BL (47 ± 8%, $p<0.05$), but were unaltered by D-glucose gavage or ADO A2b antagonism. CONCLUSION: These data suggest that NO synthase function is not chronically impaired after HS/RES. However, Na^+-linked D-glucose absorption failed to stimulate further NO production, suggesting an uncoupling of the ADO-NO signal during nutrient absorption at 24-hr post-RES.



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INTERACTIONS OF CALCIUM-CALMODULIN DEPENDENT PROTEIN KINASES(CaMK) AND MITOGEN-ACTIVATED PROTEIN KINASES(MAPK) IN MONOCYTE ADHERENCE AND TNF PRODUCTION. M Rosengart*, S. Arbabi*, I. Garcia*, R. Maier. U. of Washington, Seattle, WA 98195.

The monocyte/macrophage is recruited early after injury and orchestrates the immunoinflammatory process. Aberrant function, however, through excessive production of inflammatory cytokines such as TNF, is thought to be causal in the pathological states of MOF and shock. Since calcium is essential for cellular inflammation, we investigated the role of CaMK II and IV in LPS-induced TNF production by nonadherent and adherent monocytes. We further evaluated the adhesion-mediated enhancement of LPS-induced TNF production as it relates to CaMK-enhanced MAPK activation. **Methods:** Human monocytes were isolated by Ficoll-Paque density gradient. KN62, a general CaMK inhibitor, or autocamtide-inhibitory peptide(AIP), a CaMK II inhibitor, were applied prior to or after adherence, and cells were stimulated with LPS. Supernatant was assayed for TNF. Western blot analysis employed an antibody recognizing only active, dually-phosphorylated ERK 1/2. **Results:** Adherent monocytes produced two-fold more TNF than nonadherent cells in response to similar doses of LPS. KN62 and AIP inhibited TNF production both in nonadherent and in adherent cells that had been treated with inhibitor prior to plating. However, only KN62 inhibited TNF production if applied after adherence. Adhesion caused activation of ERK. This activation was inhibited by pretreatment with both KN62 and AIP. **Conclusion:** CaMK II, and perhaps CaMK IV, are necessary for cytokine production by nonadherent monocytes. Adhesion enhances LPS-induced production of TNF and also activates ERK. Inhibition by AIP occurs only if applied prior to adherence and correlates with ERK inhibition. This suggests that the function of CaMK II in TNF production by adherent monocytes occurs during adhesion, is mediated through ERK, and may be the event that primes monocytes for enhanced TNF production. By contrast, KN62 also inhibits TNF production in adherent cells. Thus, CaMK IV may serve a similar function during adhesion as CaMK II, but also plays a role in post-adherence cytokine production.

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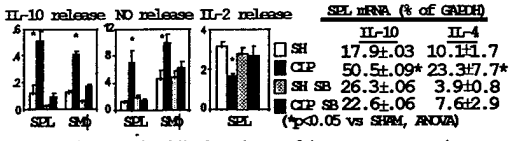
INHIBITION OF P38 MAPK ATTENUATES IMMUNOSUPPRESSION AND IMPROVES SURVIVAL IN POLYMICROBIAL SEPSIS GY Song*, CS Chung*, D Jarrar*, IH Chaudry, A Ayala. Brown Univ. Sch. Med/ RI Hospital; Providence, RI 02903.

Although studies indicate that there is a marked suppression of cell-mediated immunity during sepsis, the mechanism by which this occurs remains unknown. In this regard, studies utilizing SB203580 (SB), a specific inhibitor of the p38 mitogen activated protein kinase (MAPK) pathway, have shown that this pathway mediates inflammatory responses to septic stimuli. Although our results have indicated that p38 MAPK activation is increased in splenocytes (SPL) and macrophages (Mϕ) following sepsis, it remains unknown how p38 MAPK activation contributes to the development of immune suppression in sepsis. The aim of this study therefore was to determine the effect of p38 MAPK inhibition on immune function in polymicrobial sepsis, as well as its effects on survival. Male C3H/HeN mice underwent cecal ligation and puncture (CLP) or Sham (SH) operation. SPL and splenic Mϕ (SMϕ) were harvested 24h post-CLP and stimulated with ConA or LPS in the presence/absence of 10mM SB. IL-10, IL-2 (ng/mL,ELISA) and NO (μM,Griess Rx) release, as well as IL-10 and IL-4 gene expression (RPA) was determined (n=5-6/grp). The results indicate that IL-2 release is



SPL mRNA (% of GAPDH)

	IL-10	IL-4
SH	17.9±.03	10.1±1.7
CLP	50.5±.09*	23.3±7.7*
SH SB	26.3±.06	3.9±0.8
CLP SB	22.6±.06	7.6±2.9

(*p<0.05 vs SHAM, ANOVA)

markedly depressed, while the release of immuno-suppressive mediators, IL-10 and NO, as well as mRNA levels of IL-10 and IL-4, are augmented after CLP. Inhibition of p38 MAPK suppressed IL-10 and NO levels as well as IL-10 and IL-4 gene expression, while restoring the release of IL-2. To assess the *in vivo* effects of p38 MAPK inhibition on survival, mice (16-20/grp) were given 2mg SB/animal or saline vehicle (ip) either immediately post-CLP or 12h post-CLP. Only delayed administration of SB resulted in a marked increase in survival. Thus, these results not only illustrate p38 MAPK's role in the induction of immuno-suppressive agents encountered in sepsis, but demonstrate that SB administration beyond the pro-inflammatory, hyperdynamic state of sepsis has salutary effects on survival. (NIH GM 46354)

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LPS BINDING AND NEUTRALIZING PEPTIDE THAT MODIFIES LPS MULTIMERIC STRUCTURE AND NON-COMPETITIVELY INHIBITS LBP-LPS INTERACTION. M.J. Araña*, M. Guerra*, G. Chinea*, V. Falcon*, O. Reyes*, W. Buurman* and G. Padrón*. Center for Genetic Engineering and Biotechnology, P.O.Box 6996, La Habana, Cuba. E.mail: arana@cigb.edu.cu.

Lipopolysaccharide binding protein (LBP) is a lipid transfer protein that transfers LPS monomers from miscelles to CD14 or to HDL particles, causing cell activation or LPS neutralization respectively. Synthetic peptides based on a.a. sequences of proposed LPS-binding sites of LBP bind and neutralize LPS activities, although inefficiently. We examine the interaction with LPS of a neutralizing synthetic peptide, comprising the human LBP a.a region 86-99 (LBP_{86-99}), as well as its effects on LBP-LPS binding. This peptide decreased fluorescent-LPS binding to membrane CD14 on human monocytes and inhibited LPS-induced TNF-α release by peripheral blood cells. LBP_{86-99} effectively decreased mice mortality by peritoneal sepsis. As determined by electron microscopy, LBP_{86-99} binds to LPS micelles augmenting its radius from 19 to 29 nm and generating LPS tubular structures. The peptide inhibited LBP-LPS interaction with strong dependence on peptide:LPS ratio, but not similarly on LBP concentration, as would be expected for a competitive inhibition. Alanine scanning

was used to analyze LBP_{86-99} primary structural features contributing to LBP-LPS interaction interference. Two amino acids within the peptide are critical, while other five single residue substitutions enhanced the effect. In general, mutations decreasing net positive charge or increasing amphipathicity improved the interference. Our results suggest distinct mechanisms of interaction with LPS for the synthetic peptide and the corresponding region within the LBP protein. Identification of particular features contributing to effector functions of this peptide may aid to future developments of endotoxin antagonists for use in the treatment of Gram-negative sepsis.

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EVALUATION OF CHANGES IN GENE EXPRESSION DURING ENDOTOXEMIA. D J Brackett, M R Lerner*, G M Pighetti*, D E Branam*, J S Hanas*, E R Jupe*, and R G Postier*, Depts. Surgery and Biochem. & Molecular Biol., Univ. Okla. HSC and Okla. Med. Res. Found. Okla. City, OK 73190.

The introduction of endotoxin (ETX) into the bloodstream, either directly or as a bacterial by-product, can induce an overwhelming cascade of molecular, biochemical, and physiological responses which are not fully understood. To help elucidate the origin of these responses we have applied recently available cDNA array methodology (Clontech) to a rat model of endotoxemia. This particular array, total of 588 cDNAs, permitted simultaneous analysis of expression of gene groups associated with proteins relevant to the progression of shock, such as cytokines and their receptors, chemokines, adhesion molecules, apoptosis, coagulation, and macrophage, leukocyte, and endothelial cell function. Total RNA isolated from hepatic tissues of rats exposed to 20 mg/kg ETX or saline for 10 or 60 min was radioactively labeled and hybridized with cDNA arrays. Following exposure to phosphoimager screens the arrays were analyzed to qualitatively and quantitatively determine the changes in mRNA levels. The first-pass evaluation of these arrays focused only on determination of genes expressed in the ETX challenged tissue that were *completely absent* in the control tissues. At the 10 min time point 18 genes were expressed in the tissue from ETX challenged rats that were totally absent in the control tissue and 25 genes in the tissue taken at 60 min following ETX. Array analysis of tissue taken at 60 min revealed expression of genes associated with TNF and interleukin receptors, translation, cell surface and adhesion molecules, apoptosis, cell-cell communication, and coagulation; expression in tissue taken at 10 min was highly related to intacellular signal transduction. Gene expression related to stress response proteins, lipid and steroid metabolism and transport was found in tissue taken at both time periods. The application of this strategy, combined with the proper confirmatory assays, to molecularly dissect the progression of septic shock may define molecular mechanisms which will aid in development and assessment of therapeutic interventions for sepsis.

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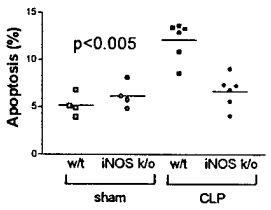
iNOS GENE DEFICIENCY DECREASES THYMOCYTE APOPTOSIS IN SEPTIC MICE. JP Cobb, RS Hotchkiss, PE Swanson*, K Chang*, Y Qiu*, VE Laubach*, TG Buchman. Washington Univ., St. Louis, MO 63110 and Univ. of Virginia, Charlottesville, VA 22908.

We hypothesized that inducible production of NO during sepsis contributes to accelerated lymphocyte apoptosis. To avoid the limitations of iNOS inhibitors, we compared the degree of thymocyte apoptosis in septic wild-type and iNOS deficient mice. The ceca of C57BL6 (control) and congenic iNOS knock-out mice were ligated and punctured. The ceca of sham animals were not injured. Thymus tissue was sampled at 18-22h. The degree of apoptosis was measured using three complementary methods: light microscopy after H&E staining; fluorescent microscopy after TUNEL staining; and two-channel FACS analysis of cells stained with annexin V (a marker of apoptosis) and propidium iodide (a negative control stain). Data were compared using ANOVA. H&E staining (N=19, not shown) revealed large inter-animal variability with regard to the degree of sepsis-induced apoptosis. TUNEL staining indicated apoptosis in cells comprising approximately 1-10% of the thymus. The average degree of apoptosis appeared to be smaller in the knock-out group (not shown). This was corroborated using quantitative FACS analysis (N=20), which showed significantly less apoptosis in iNOS knock-out animals after CLP compared to controls ($p<0.005$, see below). We conclude that inducible NO contributes to sepsis-induced thymocyte apoptosis *in vivo*. The importance of this interaction between the nitrosative and inflammatory stress responses is the focus of ongoing investigations.



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ENDOTOXIN INDUCES CROSS TOLERANCE TO GRAM POSITIVE SEPSIS. **J. Cochran*, K. Guyton*, R. Bond*, C. Romeo*, R. Southern, G. Teti* and J.A. Cook*. Depts. of Micro. and Immuno., Physiol. and Neuro., and Ped. MUSC, Charleston, S.C.**

Gram positive and gram negative sepsis share common clinical features suggesting common pathways of activation. We hypothesized that lipopolysaccharide (LPS) can produce cross tolerance (Tol) to gram positive sepsis induced by group B streptococcus (GBS). Thromboxane (TxB_2), tumor necrosis factor (TNFα), and nitric oxide (NO) production by *in vitro* LPS and GBS stimulated rat peritoneal macrophages (MØ) were measured. Tol was induced in rats by intraperitoneal injection of LPS or vehicle for two consecutive days at doses of 0.1 and 0.5 mg/kg body weight. Three days later, rats were injected i.v. with viable GBS $5x10^9$/kg and D-galactosamine 1g/kg. LPS Tol prolonged ($p<0.05$) survival time to 40.0 ± 7.0 hrs compared to 14.7 ± 2.0 hrs in non-Tol rats subjected to GBS sepsis (N=12/group). MØ from LPS Tol rats exhibited suppressed LPS induced *in vitro* TxB_2 and TNFα production ($p<0.05$). Tol also decreased ($P<0.05$) *in vitro* heat-killed GBS induced MØ TNFα production, but not MØ TxB_2 production. NO production stimulated by LPS ($p<0.05$) was not impaired by Tol and was augmented ($p<0.05$) with GBS stimulation. Our previous studies have shown that LPS Tol suppresses LPS activated extracellular receptor kinase (ERK) in MØ. In this study, PD 98059 an inhibitor of a kinase activator of ERK, blocked ($p<0.05$) both GBS- and LPS-induced TNFα and TxB_2, but not NO production. Thus, ERK activation appears essential for both GBS and LPS, induced MØ activation. These data support our hypothesis that, LPS Tol produces partial cross Tol to gram positive sepsis which suggest that common pathways of cellular activation are affected. Supported by NIH GM 27673

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INTESTINAL PERMEABILITY AND NEUTROPHIL INFLUX INTO ILEA OF LPS-TREATED MICE. S. Debol*, R. Jechorek*, K. Kinneberg*, J. Hoag*, B. Feltis*, S. Erlandsen*, C. Wells. Univ. of MN, Minneapolis 55455.

Endotoxin-mediated septic shock is characterized by increased intestinal permeability and neutrophil influx into the intestinal lumen. To determine if neutrophil migration is associated with altered intestinal permeability, mice were given 75-150 µg i.p. *E. coli* lipopolysaccharide (LPS). To visualize the intestinal barrier, 5 mg horseradish peroxidase (HRP, a paracellular tracer) was injected into the vena cava, ileal tissue was excised 3 min later, and the brown reaction product (developed with diaminobenzidine) was visualized by light microscopy. In control mice, HRP was localized between enterocytes up to the tight junctions and not in the intestinal lumen; 1 hr after LPS, HRP was seen on apical enterocyte microvilli and in luminal contents, indicating opening of tight junctions and paracellular HRP passage. To quantify HRP passage, mice were given 10 µg oral HRP, and serum HRP was quantified 30 min later using mouse anti-HRP IgG in an ELISA antigen-capture assay; serum HRP was 0.2±0.4 ng/ml (avg±SD) in control mice and increased ($P<.05$) to 1.0±2.0 and 1.3±2.2 ng/ml in mice sacrificed 6 and 16 hr after LPS. To assess neutrophil influx into tissue, myeloperoxidase (MPO) was extracted from excised lung and ilea (8 cm) and measured by colorimetry following oxidation of tetramethylbenzidine. Lung MPO increased from 1.5±1.2 Units/g in control mice to 15.0±0.7 U/g 1 hr after LPS ($P<.01$), and remained high throughout this 48 hr experiment. Ileal MPO, consistently less ($P<.01$) than lung MPO, increased from 0.6±0.6 U/g in control mice to 0.7±0.3 and 1.3±1.0 U/g in mice sacrificed 6 and 48 hr after LPS ($P<.01$). All data were highly variable, and were analyzed by Kruskal-Wallis with Mann-Whitney post hoc test. Thus, assays with HRP indicated that intestinal permeability was altered 1 hr after LPS and remained elevated for 16 hr. MPO measurements indicated that influx of ileal neutrophils was comparatively modest compared to lung neutrophils, with ileal MPO maximal 48 hr after LPS, a time when the lumen was densely filled with leukocytes as visualized by light microscopy of stained ileal sections. Thus, neutrophil influx into the intestinal lumen may be associated with LPS-induced increase in intestinal permeability.

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THE EFFECTS OF *IN VIVO* PRETREATMENT WITH IL-1 RECEPTOR ANTAGONIST, ROLIPRAM OR DMSO ON *EX VIVO* RAT AORTIC CONTRACTILITY. LL Donaldson*, AK Myers* (spon: CM Otto) Georgetown University, Washington, DC 20057

The pathophysiology of septic shock includes clinically recognizable vascular motor dysfunction that can be simulated by *in vivo* exposure to lipopolysaccharide (LPS), interleukin-1 (IL-1) and tumor necrosis factor (TNF) and demonstrated by conventional *in vitro* isometric contractility techniques. Using a sublethal model of endotoxemia, an attempt was made to assess the contributions of IL-1 and TNF on LPS-induced vasoplegia by blocking an IL-1 receptor and suppressing the release of TNF. The IV administration of 5 mg/kg *Escherichia coli* (0127:B8) LPS to male Wistar rats resulted in hypotension, tachycardia, non-significant increases in serum IL-1 and corticosterone, a significant increase in serum TNF at 2 hours and reduced *ex vivo* responses of aortic rings to phenylephrine at 6 hour. Treatments consisted of 5 mg/kg IL-1 receptor antagonist (IL-1ra) 15 minutes before and 3 hours after LPS, 100 ug/kg of the phosphodiesterase inhibitor, rolipram, 15 minutes before LPS or equal volumes of their vehicles, citrate buffer and dimethyl sulfoxide (DMSO), respectively. Pretreatment with rolipram reduced peak TNF levels 2 hours after LPS. IL-1ra, rolipram and DMSO (0.06 mg/kg) preserved the enhanced sensitivity to PE that is associated with removal endothelium from healthy aortic rings. Neither IL-1ra nor rolipram prevented the decrease in maximal contractile tension produced by LPS but endothelium denuded aortic rings removed from rats treated with DMSO contracted to tensions similar to those of aorta from control rats. Efforts to manipulate the inflammatory response to LPS altered *ex vivo* aortic contractility in this sublethal model.

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EFFECT OF *CLOSTRIDIUM DIFFICILE* TOXINS A AND B ON INTERACTIONS OF *E. COLI* WITH CULTURED ENTEROCYTES. BA Feltis*, AS Kim*, K Kinneberg*, DL Lyerly*, TD Wilkins*, SL Erlandsen*, CL Wells. Univ. of MN, Mpls, 55455;VPI & State Univ., Blacksburg, VA 24060.

Clostridium difficile toxins A and B are widely recognized as etiologic agents of antibiotic associated diseases ranging from diarrhea to pseudomembranous colitis. These toxins cause actin cytoskeletal collapse and alter junctional permeability in cultured enterocytes. Critically ill patients are at high risk for antibiotic-induced *C. difficile* disease, as well as systemic infection caused by enteric bacteria. Therefore, experiments were designed to clarify the effects of purified toxins A and B on bacteria-enterocyte interactions. Mature HT-29 enterocytes were preincubated 16h with toxin A or B (0.01 to 100 ng/ml) followed by 1h incubation with 10^8 *E. coli*. HT-29 viability was unaffected (using vital dyes), and numbers of internalized *E. coli* were increased in the presence of toxin A but not B. Epithelial permeability was assessed by bacterial migration through confluent enterocytes cultivated on permeable filter supports; toxin A but not B, was associated with increased bacterial migration through enterocyte monolayers.

C. difficile toxin (ng/ml)	Avg±SE log10 internalized *E. coli*	Avg±SE log10 migrating *E. coli*[a]
A (0)	1.7 ± 0.1	2.9 ± 0.8
A (10)	1.7 ± 0.1	5.8 ± 0.2*
A (100)	2.3 ± 0.2*	6.0 ± 0.2*
B (0)	1.7 ± 0.1	4.1 ± 0.4
B (1)	1.7 ± 0.1	4.0 ± 0,6
B (100)	1.8 ± 0.1	4.3 ± 0.3

a. Number of bacteria recovered from basal chamber 1h after 8.0 log10 added to apical chamber; *$p<0.01$ vs 0 ng/ml

Thus, *C. difficile* toxin A was associated with increased transepithelial migration of *E. coli* as well as enterocyte uptake of *E. coli*. Thus, intestinal colonization with *C. difficile* may play a heretofore unrecognized role in augmenting bacterial migration across the intestinal epithelial barrier.

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SECRETORY LEUKOCYTE PROTEASE INHIBITOR, AN INHIBITOR OF NEUTROPHIL ACTIVATION, IS ELEVATED IN SERUM IN HUMAN SEPSIS AND CORRELATES WITH SEVERITY OF ORGAN DYSFUNCTION. S. Grobmyer, P. Barie, C. Nathan*, M. Fuortes*, E. Lin, S. Lowry, C. Wright*, M. Weyant*, L. Hydo*, F. Reeves*, M. Shiloh*, A. Ding*. Weill Med. Coll. of Cornell U., NY, NY 10021 and Robert Wood Johnson Med. Sch., New Brunswick, NJ 08903

Secretory leukocyte protease inhibitor (SLPI) has recently been shown *in vitro* to be induced by bacterial cell wall components, lipopolysaccharide and lipotechoic acid, and to have immunomodulatory functions. In order to assess if SLPI may be involved in the human systemic inflammatory response *in vivo*, we measured SLPI levels in human experimental endotoxemia and in human sepsis. We found a significant ($p = 0.01$) and dose dependent elevation in

plasma SLPI following administration of lipopolysaccharide to healthy adults. Further, we noted serum concentrations of SLPI were elevated in septic surgical patients compared to healthy controls ($p < 0.01$) and non-septic surgical controls ($p = 0.02$). Serum SLPI concentration correlated ($r = 0.84$, $p < 0.01$) better with organ dysfunction as measured by maximal multiple organ dysfunction (MOD) score than did serum IL-6 ($r = 0.70$, $p<.0.01$), IL-10 ($r = 0.23$, $p = 0.22$), or TNF-α ($r = 0.14$, $p = 0.44$). Neutrophil mediated tissue damage has been implicated in the pathogenesis of multiple organ failure. We found recombinant human SLPI (rhSLPI) inhibits adherent neutrophil production of hydrogen peroxide (IC 50 = 250 ng/ml). SLPI therefore is a component of the human systemic inflammatory response and may function in part to help limit ongoing neutrophil mediated tissue injury that is assoicated with organ dysfunction.

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ANALYSIS OF THE INFLAMMATORY RESPONSE INDUCED BY LAPAROSCOPIC CECAL LIGATION AND PUCTURE. E.J. Hanly*, M. Mendoza-Sagaon*, K. Murata*, J. M. Hardacre*, M.A. Talamini* and A. De Maio.
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Unintentional bowel injury is a possible complication during laparoscopic abdominal procedures. The response to such injury may be complicated by factors associated with the laparoscopic procedure such as insufflation by CO2. In the present study, sepsis induced by cecal ligation and puncture (CLP) performed by a laparoscopic procedure was compared to CLP performed after laparotomy. Female Sprague-Dawley rats were fasted for 16 h prior the procedures. Animals were anesthetized with methoxyfluorane and randomized into five groups. One set of rats was anesthetized without operative manipulations. Another group of rats was subjected to laparoscopic CLP (LCLP). The controls for this procedure were rats that were insufflated with CO2 without surgery. Another group of rats underwent an open procedure consisting of a midline laparotomy followed by CLP (OCLP). The final group of animals was subjected to laparotomy without CLP. CLP was induced by ligation of the cecum and double puncture with a 16G needle. Animals were resuscitated with a subcutaneous injection of ringer's lactate (30ml/kg). Rats had access to water ad libitum after the respective procedure. No differences in mortality were observed between rats after LCLP and OCLP within 48 h of the procedure. Serum levels of interleukin 1α and tumor necrosis factorα as well as the expression of acute phase genes within the liver were also similar after 24 h of LCLP and OCLP. The number of leukocytes in blood samples was significantly reduced in rats that underwent OCLP with respect to animals that were subjected to LCLP. In general, our results do not show a major difference in the inflammatory response after CLP induced by open or laparoscopic procedures.

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TRAUMATIC AND SEPTIC SHOCK. R. M. Hardaway, C. H. Williams*, and Y. Vasquez*, Texas Tech Univ. HSC, El Paso, TX 79905.

A theory of traumatic and septic shock is presented. It postulates that both traumatic and septic shock are accompanied by DIC, which temporarily occludes the microcirculation of any or all organs, temporarily cutting off the circulation to those organs and causing MOF. DIC may be brought on by rupture of the walls of red cells, tissue cells, bacteria, or all three. Red cells and tissue cells may be broken by trauma, cold or heat, anoxia, viruses or plasmodia. Bacterial cell walls may be broken by antibiotics, heat or antibodies. The inner layer of all cell walls consists of thrombogenic aminophospholipids. When cells are broken, this thrombogenic phospholipid may initiate DIC, acting as an autotoxin. DIC may block the microcirculation of any or all organs, causing ARDS and MOF. It is postulated that the polysaccharide endotoxin is not the main initiator of septic shock, but that thrombogenic phospholipids are. Microclots of DIC in an organ's microcirculation may be lysed by a plasminogen activator. Lysing of microclots in the lung restores the microcirculation in that organ and effectively treats DIC. Bleeding has not been caused in the dosage described. The administration of urokinase for the treatment of traumatic or septic shock in pigs is both safe and effective. Its administration for the treatment of septic or traumatic shock in humans has proved safe in a phase I study. The effectiveness of this agent in the treatment of septic shock or traumatic shock must await a prospective randomized study, which is already under way in 20 centers.

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INCREASED RELAXATION RATE PERMITS MEASUREMENT OF STABLE END-DIASTOLIC PRESSURE-DIMENSION RELATIONS IN ENDOTOXEMIA G.M.Jacobson*, L.J.Segil*, R.L.Ripper*, and W.R. Law Departments of Surgery, Anesthesiology, and Physiology and Biophysics, Univ. of Ill. at Chicago, 60612

Tachycardia is common in septic shock, and we have reported that at heart rates (HR)>125, the left ventricle does not reach a stable, minimal diastolic pressure-dimension relationship (DPDR) prior to viscoelastic influences of left atrial contraction (LAC). The present work was performed to determine if similar findings were asspciated with endotoxin-induced tachycardia. Anesthetized pigs (25-30 Kg) were instrumented to obtain high fidelity LV pressure and minor axis circumference (sonomicrometry). After baseline measurements of variably loaded cardiac cycles (vena cava occlusion) at increasing HRs, pigs received iv E. coli endotoxin (500 $\mu g \cdot kg^{-1}$), and measurements were repeated. The relaxation time constant, tau, was calculated. The DPDR was determined from 10 msec increments of isochronal data 0-60 msec prior to LAC fit to a monoexponential relationship. Significance ($p<0.05$) was determined by t-test (tau) or ANOVA (regression coefficients). Prior to endotoxin, the LV DPDR did not reach a stable relationship prior to LAC when HR was >125. After endotoxin, tau increased significantly (22 ± 3 to 30 ±4 msec), indicating increased rate of relaxation. As a result, LV DPDR reached a stable relationship at least 30 msec prior to LAC after endotoxin administration at HR up to 195. The coefficients of the LV DPDR after endotoxin indicated a significant shift to the left, suggesting a decrease in LV compliance. These results indicate that an increased rate of LV relaxation during endotoxemia permit accurate measurement of the LV DPDR despite tachycardia. The left shift of the LV DPDR at a time when LV relaxation rate is increased suggests that changes in relaxation rate cannot predict end-diastolic compliance during septic shock. Support: VA Merit Review, and Department of Surgery and Anesthesiology.

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THE EFFECT OF TNF ALPHA AND IL-1 BETA ON THE RELATIONSHIP BETWEEN SYSTEMIC OXYGEN DELIVERY AND CONSUMPTION. Y. Kuwagata*, J. Oda* and H. Sugimoto*, (Spon: H. Hirasawa). Osaka Univ. Med. Sch., Osaka 565-0871, Japan.

As systemic oxygen delivery (DO_2) is reduced, oxygen consumption (VO_2) is maintained until a critical level is reached (DO_2crit). Sepsis is thought to shift DO_2crit to the right and lengthen the supply-dependent portion. We tested the effect of tumor necrosis factor alpha (TNFα) and interleukin-1β (IL1β), the key cytokines of sepsis, on DO_2-VO_2 relationship. Fifteen rabbits were randomly divided into three groups (n = 5, each) and given 10 μg of TNFα or 10 μg of IL1β or saline (Ctrl) intravenously. All rabbits were subjected to stepwise cardiac tamponade to reduce DO_2 down to 10% by inflating a handmade balloon placed into the pericardial sac. The DO_2-VO_2 relationship was analyzed by the dual line method. IL1β significantly decreased mean arterial pressure (62 ± 7 mmHg from baseline 83 ± 5 mmHg) without altering cardiac output while TNFα had no significant effect on hemodynamics. IL1β group showed significantly steeper slopes of the supply-independent portion than TNFα group or Ctrl (IL1β group: 0.19 ±0.02, TNFα group: 0.03 ±0.10, Ctrl: 0.11 ± 0.02) which resulted in shifting DO_2crit to the left (IL1β group: 8.4 ± 1.5 ml/kg/min, TNFα group: 12.4 ± 1.4 ml/kg/min, Ctrl: 11.6 ± 1.0 ml/kg/min). IL1β group also showed upward shift of the DO_2-oxygen partial pressure relationship in the portal circulation which was not seen in TNF group or in Ctrl. These results indicate that IL1β impairs systemic oxygen uptake even before VO_2 becomes supply-dependent presumably due to the maldistribution of blood flow to the splanchnic circulation.

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THE COMBINATION OF APACHE II-SCORE WITH SIRS/SEPSIS-CRITERIA PREDICTS OUTCOME IN SURGICAL ICU PATIENTS MORE PRECISELY THAN APACHE II ALONE.

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Objective: To evaluate if the combination of APACHE II-score with SIRS/Sepsis-criteria is a more accurate predictor of mortality than APACHE II-score alone.

Design: Prospective study.

Patients and Methods: All surgical ICU patients (n=689) in 1998 were classified into three groups: a) patients without SIRS or sepsis (control); b) patients with at least two SIRS-criteria; and c) septic patients. For each patient demographic and hospital survival data were raised and SIRS-, sepsis- and APACHE II scores were calculated. The mean values of the survivors were compared with the non-survivors in each of the three groups. The accuracy in mortality prediction was assessed by means of the ROC curve.

Results: The mean APACHE II-score in the control- and SIRS-group was significantly higher ($p < 0.001$) in the non-surviving group versus survivors (9.91 vs. 18.33 and 11.52 vs. 21.33). SIRS criteria are associated with a moderate sensitivity (0.73) and specificity (0.65) in predicting the outcome. APACHE II-scoring shows a sensitivity of 0.85. The combination of the scores is most sensitive in predicting outcome of SICU patients. The discriminating power of the combination, measured by the area under the ROC curve was excellent (0.89) and higher compared to APACHE II alone (0.87).

Conclusion: Our results suggest that the combination of APACHE II-score with SIRS/Sepsis criteria allows a more precise prediction of mortality than APACHE II alone.

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PERITONEAL ABSCESS FORMATION INDUCES LUNG IL-6 MACROPHAGES AND ARDS-LIKE ABNORMALITIES IN SEPSIS. J.R. Lussier*, N. Espina*, L. Macedo*, L. Quinn*, C. Idler*, and J.H. Siegel, Department of Anatomy, Cell Biology, and Injury Sciences, UMDNJ- New Jersey Medical School and Graduate School of Biomedical Sciences, Newark, NJ 07103.

The development of a severe septic process is frequently associated with the subsequent evolution of the acute respiratory distress syndrome (ARDS). This study examines the relation of abscess development in a septic rat model, with the onset of ARDS-like changes in the lungs. It employs an animal model in which a 1.5cc sterile fecal-agar pellet, either alone or contaminated with 10^2 *E.coli* and 10^8 *B.fragilis*, is implanted into the peritoneal cavity of a rat. This reproducible model presents with an acute peritonitis phase on day 1, which is then followed by development of a chronic sterile or septic abscess. The pattern of septic response, and its evolution was studied over time. Characterization of fibrin deposition and collagen synthesis, to form the abscess, was examined by H&E, and Gomori's Trichrome staining procedures. Interleukin-6 (IL-6), which is known to induce hepatic fibrinogen (the precursor of fibrin) synthesis, was detected by its mRNA in lung tissue macrophages (mϕ) by *in situ* hybridization, with visualization by confocal microscopy. Lung tissue analysis demonstrated an increased IL-6 mϕ mRNA expression in the sterile inflammation and septic lungs as compared to controls, with a subsequently greater increase in the septic animals at days 2 and 3, as the abscess formed. The pattern of ARDS was evident in the lung with increased fibrin and collagen deposition in the interstitium, associated with increased mϕ infiltration. The IL-6 mRNA expression in lung mϕ's corresponds to peak cytokine levels in circulation at day 3. We propose that the abscess plays a key role by acting as a functional chemokine-inducing organ, which results in a large lung infiltration of IL-6 producing mϕ's, during chronic septic inflammation with enhanced ARDS-like changes.

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MAPPING OF GENES THAT MODULATE THE INFLAMMATORY RESPONSE INDUCED BY ENDOTOXIN. L. E. Matesic*, E. L. Niemitz*, A. De Maio, and R. H. Reeves*.
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The incidence of multiple organ dysfuction syndrome (MODS) is still very high in patients after an episode of trauma, sepsis, pancreatitis or severe burns. The current dogma posits that MODS is the result of an overwhelming inflammatory response. Previous studies have indicated that the inflammatory response is likely genetically modulated. Two inbred strains of mice, C57BL/6J (B6) and A/J, showed differences in mortality, circulating cytokines levels and expression of acute phase genes within the liver after administration of *E. coli* lipopolysaccharide (LPS). In addition, B6 mice showed a greater number of infiltrating polymorphonuclear leukocytes (PMNL) in the liver 24 hours after injection of LPS (15 mg/kg) as compared to A/J mice. The average

number of PMNL observed per high power magnification field was 51.05±15.51 in B6 mice as compared to 13.76±5.31 in A/J mice ($p=6.4X10^{-20}$). Analysis of the latter phenotype in reciprocal F_1 progeny and in a small number of backcross mice (n=27) suggests that this response is controlled by multiple autosomal genes. To map these genes, the inflammatory responses of mice from 28 AXB/BXA recombinant inbred strains were analyzed. Three loci that contribute to the observed hepatic PMNL infiltration (*Hpi*) were mapped to mouse chromosomes 5, 10 and 17. The *Hpi* loci are localized within 7 cM to 15.3 cM (as compared to the 1500 cM mouse genome). Candidate genes, including *TNF-α*, *TNF-β* and *IL-6*, which map to these regions of the genome, are currently being evaluated for genetic differences between B6 and A/J mice. The human homologues of these candidate genes can be identified by comparative mapping, allowing us to study their possible contribution to the development of MODS.
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ACUTE ENDOTOXIN SHOCK ALTERS THE PHARMACOKINETICS OF LIDOCAINE AND ITS METABOLITE, MEGX.
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The metabolism of lidocaine (LC) to MEGX has been used as a marker of hepatic function. A previous investigation determined that the formation of MEGX from LC was unchanged in the *in situ* isolated perfused liver during acute endotoxin shock in the rat. The present study was undertaken to evaluate the pharmacokinetics of LC and MEGX in an *in vivo* acute endotoxin shock rat model. Male Sprague-Dawley rats were assigned to endotoxin (46 mg/kg, i.p.; n = 7) or control (n = 5) groups. Endotoxin was administered 6 hours prior to LC administration. Approximately 45 min prior to LC administration, rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and the trachea and carotid artery were cannulated. LC (2mg/kg) was administered to all rats followed by serial blood samples collected up to 2 h following LC administration for determination of LC and MEGX serum concentrations. Arterial blood pressure (BP) was recorded throughout the experiment. BP was significantly lower in the endotoxin treated rats. LC (1.16±0.52mg/L vs. 0.05±0.03mg/L) and MEGX (627±123μg/L vs. 138±44μg/L) concentrations were significantly greater in the endotoxin vs. control group 120 min following LC administration. These data demonstrate that the elimination of LC is impaired following shock. The elevated serum LC concentrations are the result of significant hepatocellular dysfunction since BP changes alone could not explain the altered pharmacokinetics. The elevated MEGX concentrations suggest a preferential metabolism of LC to MEGX and/or reduced elimination of MEGX.

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ESTROGEN AS A MEDIATOR OF *VIBRIO VULNIFICUS* LPS-INDUCED ENDOTOXIC SHOCK
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A sexually dimorphic response is observed when LPS isolated from Vibrio vulnificus, a gram-negative estuarine bacterium, is injected into mature Sprague-Dawley rats. We hypothesize that this sexually dimorphic response is mediated by estrogen through alterations in paracrine regulators of immune and cardiovascular responses. To determine the influence of estrogen on the progression of endotoxic shock, gonadectomized animals were treated with or without estrogen (2μg/day in corn oil) for five days followed by a single injection of LPS on day five. Both gonadectomized males and females treated with estrogen had significantly lower mortality rates than normal males following estrogen treatment ($p<0.05$). However, the mortality rates of the estrogen-treated animals were not different from normal females in estrus. To determine if estrogen mediates the immune response, which may result in protection against endotoxemia, expression of TNF, IL-6, IL-12 and IL-18 mRNAs from isolated peritoneal macrophages (MACs) was measured by RT-PCR. Preliminary results suggest that male MACs preincubated with estrogen show a dose-dependent decrease in TNF expression in the absence and presence of LPS whereas female MACs show no change in TNF expression. IL-6 and IL-12 expression in female MACs increases with LPS, while no change in expression is observed in estrogen-treated male MACs when treated with LPS. IL-18 mRNA expression decreases dose-dependently in estrogen-treated female MACs in the absence of LPS and increases in the presence of LPS. Estrogen-treated male MACs show no change in mRNA expression in the absence or presence of LPS. Mortality studies show that estrogen decreases mortality in *Vibrio vulnificus* LPS-induced endotoxic shock. In addition, estrogen-treated male and female MACs show sexually dimorphic patterns of TNF, IL-6, IL-12 and IL-18 mRNA expression. NIH AI37822

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INDOMETHACIN OR IL-10 NEUTRALIZATION CAN REVERSE THE PROTECTIVE EFFECT OF TNFα PRETREATMENT AGAINST ENDOTOXEMIA
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We have previously shown that pretreatment with small amounts of TNFα can attenuate shock and mortality in swine and murine models of septic shock. To explain these protective effects, we hypothesized that TNFα up-regulated endogenous mediators that might suppress subsequent pro-inflammatory activity. Specifically, we hypothesized that PGE2 and/or IL-10 might be induced by TNFα and would suppress the response to further inflammatory stimulus. To test this hypothesis, BALB-c mice (10-12 weeks, ~20g) were injected intraperitoneally (IP) with recombinant murine TNFα at a dose of 50ug/kg in 0.1ml bovine serum albumin (BSA). Mice were also given injections of indomethacin (5mg/kg IP) 1hr prior to TNF, or neutralizing anti-IL-10 antibody (200ug) 18hrs after TNF. Twenty-four hours after the TNFα injection, mice were injected with a highly lethal dose of endotoxin (E. coli 0111:B4, 6.25mg/kg). Mortality was recorded for the 48-hour period following endotoxin injection.

Results: Mice Survival after Endotoxin Administration

Pretreatment Group	Survival	Significance
1.BSA (control)	8/21	
2.TNF in BSA	16/19	p<.01 vs. #1
3.TNF + anti-IL-10	3/8	p<.05 vs. #2
4.TNF + isotype Ab	8/8	
5.TNF + Indometh	1/5	p<.05 vs. #2
6.Indomethacin alone	4/5	

Conclusions: TNFα pretreatment was associated with a significant attenuation of mortality during subsequent endotoxemia and may be explained partially by up-regulation of endogenous anti-inflammatory mechanisms, including IL-10 and cyclooxygenase products. Elucidation of these mechanisms may lead to potential therapies for patients suffering, or likely to suffer, septic shock.
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DEHYDROEPIANDROSTERONE IMPROVES SURVIVAL RATE AND IMMUNE FUNCTION AFTER POLYMICROBIAL SEPSIS. Oberbeck, JR+, Dahlweid, FM*, Koch, R*, Pape, HC*, Tscherne, H*. +Dept. Trauma Surgery, University of Essen, 45147 Essen, Germany, *Dept. Trauma Surgery, Hannover Medical School, 30625 Hannover Germany (Spon: H-J Oestern, Germany).

Sepsis is a common complication in patients suffering from severe trauma. We investigated the effect of the steroidhormone Dehydroepiandrosterone (DHEA) (40mg/kg sc.) on the mortality rate and on cellular immune functions in NMRI mice 48h and 96h following induction of polymicrobial sepsis by cecal ligation and puncture (CLP) (n=15) or laparotomy (sham) (n=15). Control animals (controls) were treated with saline injection following CLP (n=15) or laparotomy (n=15). Cellular immune function was determined by evaluation of the delayed type of hypersensibility reaction (DTH) on the basis of pinna swelling after Dinitrofluorobenzene (DNFB) administration. Furthermore, immune cell migration (CD4, CD8, CD56) was monitored. DHEA administration significantly decreased the mortality rate and increased cellular immune functions 48h and 96h after CLP (vs. controls). This was accompanied by an increase of blood monocytes and a decrease of circulating CD56+-cells in DHEA-treated animals 96 h after CLP.

	pinna-swelling ratio	48h-mortality (%)
A: CLP/saline	1.02±0.03*	35** (vs. C)
B: CLP/DHEA	1.38±0.14* (vs. A)	19** (vs. A)
C: Sham/saline	1.45±0.11	0
D: Sham/DHEA	1.40±0.09	0

Statistics: Wilcoxon-Test; *p<0.01, **p<0.001

DHEA increased the survival rate after polymicrobial sepsis in mice. Furthermore, a normalisation of DTH-reaction and alterations of immune cell migration were observed. Our findings indicate a beneficial effect of DHEA in systemic inflammation.

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EFFECTS OF HUMAN ANTITHROMBIN III ON RAT CECAL LIGATION PUNCTURE SHOCK.

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To evaluate the effects of antithrombin III (ATIII), on septic shock, male Wister rats were subjected to shock induced by cecal ligation hpuncture (CLP). Following laparotomy under light ether anesthesia, the cecum was ligated below the ileocecal valve and two holes were made by 18 gauge needle. Mortality and mean survival time were monitored, and plasma 6-keto prostaglandin F1α (6-keto PGF1 α) and thromboxane B2 (TXB2) were also measured at four hours after CLP. Animals were devided into sham and septic groups treated with or without ATIII 100 U/kg (intravenous injection, i. V.). All sham and sham with ATIII rats survived for more than 48 hours. Animals subjected to CLP (n=8) exhibited severe shock with a mean survival time of 12.6 ± 3.8 hours. Animals treated with ATIII (iv) simultaneously with CLP (n=8) significantly (p<0.01) prolonged survival to 31.3 ± 3.4 hours and 50% of rats survived. Serum concentration of 6-keto PGF1 α was increased from sham controls, ATIII significantly decreased the production. CLP also increased the production of TXB2 while ATIII again decreased the production. These results suggest that ATIII may have beneficial effects on CLP shock, possibly by blocking inflammatory mediator production.

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EFFECTS OF RED BLOOD CELL TRANSFUSIONS ON MORTALITY IN SEPTIC PATIENTS.

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Introduction : The role of transfusions and the optimum haemoglobin concentration remain unclear in septic conditions where rheologic abnormalities are a common event. Between January 1998 to September 1998,we analyzed in a retrospective study, the effect of Red Blood Cell (RBC) transfusions on mortality in a septic population. **Methods** . During this period, we compared major risk-factors and mortality in population of transfused (T) and non-transfused (NT) septic patients

(respectively n=99 and n=25 patients). Risk-factors studied were the type of admission divided in three great categories : traumatic , hemorrhage or surgical admission. We compared also the age of patients and the SAPS 2 score in these two population. **Results** :Table 1.

	T. (n=99)	N.T. (n=25)	P values
SAPS 2 score	44	39	0.013
Median age	69	67	0.27
Traumatic	13	0	0.158
Surgery	50	11	0.884
Hemorrhage	14	0	0.136
Mortality	36	7	0.739

We observed a significant association with SAPS 2 score (p=0.013), who is higher in transfused population than in non-transfused population.No significantly modifications were demonstrated with other risk-factors: type of admission and age.Despite a higher SAPS 2 score, no effect on mortality was observed in the transfused septic population. **Conclusions** : In this small retrospective study, RBC transfusions does not affect the mortality in septic patients despite a higher SAPS 2 score in this particular population. These data suggest a possible benefict effect of RBC transfusions on sepsis treatment with replacement of previously rigidified RBC by cells with a normal deformability.

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GENETIC INFLUENCE ON THE RESPONSE TO SEPSIS.

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Despite recent advances in the management of critically ill and injured patients, the complexity of events following injury remains unclear. Thus, it is impossible to predict which patient will progress to multiple organ dysfunction syndrome. We have postulated that the clinical outcome to injury is a result of multiple factors including the environment, physical condition, and the genetic make up of the individual. Previous studies have shown significant differences in mortality and inflammatory response between two genetically different strains of mice, C57BL/6J (B6) and A/J mice, after administration of *E. coli* endotoxin. In the present study, the response of these two mouse strains was compared

after polymicrobial fecal peritonitis induced by cecal ligation and single puncture (CLP). Adult (eight - week old) male B6 and A/J mice were maintained in an identical pathogen-free animal facility and fasted for 16 hours prior to the procedure. Anesthetized mice underwent CLP (25G needle), followed by resuscitation with 1.0cc of normal saline with access to water and food ad libitum. Mortality was significantly higher in B6 mice (34/38, 89.47%) with respect to A/J mice (19/41, 46.3%) during a period of 48 hours after CLP (p=0.001). The circulating levels of TNF-α, IL-1β, IL-6 and IL-10 increased after CLP in both mouse strains, and no differences were observed between them. However, expression of β-fibrinogen and metallothionein in the liver was higher in A/J mice as opposed to B6 mice after CLP. These data support the hypothesis that the response to sepsis may be genetically modulated. Genetic analysis in a mouse model will allow us to search for potential loci contributing to this disparate response to sepsis which could be extrapolated to humans. This information may ultimately have an effect on the management of critically ill patients. Supported by NIH grant GM57317 and the Robert Garrett Research Foundation.

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MONOCYTES MANIFEST REPROGRAMMED CYTOKINE RESPONSES IN *E.COLI*-INFECTED PRIMATES. A. Shnyra*, R. Brewington*, E. Zuvanich*, D. C. Morrison, A. C. K. Chang, G. Kinasewitz, G. Peer*, and F.B. Taylor. Univ. Kansas Med. Center, Kansas City, KS, St. Luke's Hospital, Kansas City, MO, and Oklahoma Med. Res. Found., Oklahoma City, OK.

Pharmacological interventions to date showed low efficacy in treatment of sepsis and septic shock. Failure to improve the outcome in sepsis may well result from inappropriate use of these innovative therapies designed to target the pro-inflammatory phase of sepsis. Using a fibrin clot model of *E.coli* infection in baboons, we experimentally investigated the existence of interrelated and reciprocal induction of pro- and anti-inflammatory phases of the immune response in sepsis. Our results indicate that *E.coli* infection in primates is associated with a transient state of endotoxemia that reaches a maximum at 24h post infection. Circulating levels of TNF-alpha, IL-6, IL-8, IL-10 and IL-12 were differentially upregulated during the first 2-48h after infection. Expression of cytokine mRNA, measured by RT-PCR in PBMC isolated from infected baboons at different times, strongly suggest a reciprocal modulation of pro-inflammatory (TNF, IL-12, IL-18) and anti-inflammatory (IL-10) cytokine responses. These data show that Gram-negative sepsis induces an early (2-6h) and late (48-72h) pro-inflammatory phases of innate immune responses, while the anti-inflammatory phase is dominant at 24h after infection. Interestingly, elevated IL-10/IL-12 ratios of cytokine mRNA assessed at 6-24h were predictive with respect to survival of infected primates. In contrast, death of experimental animals usually occurred during the late pro-inflammatory phase (48-72h) and was associated with low IL-10/IL-12 ratios of cytokine mRNA shown at 6-24h. Our data provide strong experimental support for the hypothesis that both pro-inflammatory and compensatory anti-inflammatory phases of the innate immune response are reciprocally modulated in sepsis, as assessed by reprogrammed cytokine potential in monocytes/macrophages. We conclude that new therapeutic strategies, either pro- or anti-inflammatory, should target the corresponding immuno-inflammatory phase of the host response in order to improve their efficacy and outcome in sepsis and septic shock.

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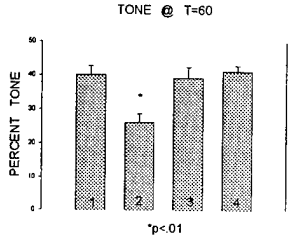
PDTC, AN INHIBITOR OF NF-κB, BLOCKS ENDOTOXIN INDUCED VASODILITATION OF ISOLATED RAT SKELETAL MUSCLE ARTERIOLES. J. Snyder*, R.Prewitt* and L.D. Britt Eastern Virginia Medical School, Norfolk, VA 23507

NF-κB is a ubiquitous transcription factor that mediates the inflammatory response. The antioxidant Pyrrolidine Dithiocarbamate has been shown in previous work to selectively inhibit NF-κB activation. The goal of this study was to determine if PDTC could inhibit resistance arteriole vasodilitation in response to endotoxin. Male Sprague Dawley rats were given an i.p. injection of PDTC, 100 mg/kg, or a sham injection of saline (N=21). First order cremasteric arterioles were isolated, cannulated and pressurized to 70 mmHg. A segment of thoracic aorta was then placed in series with the microvascular preparation. This in-series model has demonstrated a significant loss of arteriolar tone in response to endotoxin during previous experiments. Vessels were allowed to equilibrate and achieve spontaneous myogenic tone in a bath of warm physiologic buffer over 1 hr.(t=0). Internal vessel diameters were measured with video calipers and the response to endotoxin(ET) or continued infusion of buffer was measured over one hour, (t=60). Group 1(n=6) was a time control group, receiving a sham injection and being exposed to buffer only. Group 2(n=6) was exposed to ET only, Group 3(n=5) received PDTC and ET while group 4(n=4) received PDTC only. Spontaneous tone (measured as a percent of maximal diameter) was similar in the four vessel groups at the end of the equilibration period (t=0). After 1 Hr. (t=60), Group 2 (vessels exposed to ET only) had significantly less tone (26.1±2.6%; p<.01) than group 1 (40.3±2.5%), group 3 (39.3±3.1%) and group 4 (41.2±1.6%). We conclude that PDTC, an inhibitor of NF-κB, blocks endotoxin induced vasodilitation in isolated rat skeletal muscle arterioles.



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A RELATIONSHIP EXISTS BETWEEN DECREASED ILEAL Na^+,K^+-ATPASE ACTIVITY AND BACTERIAL TRANSLOCATION AFTER ENDOTOXIN CHALLENGE
Y. Suzuki*, D. Xu, Q. Lu*, E. Deitch, UMDNJ-New Jersey Med. Sch., Newark, NJ, 07103

Introduction: We have previously shown that LPS and NO inhibit Na^+,K^+-ATPase in cultured rat intestinal epithelial cells and that iNOS knockout mice are resistant to LPS-induced bacterial translocation (BT). Since LPS-mediated BT could be related to NO-induced decreased Na^+,K^+-ATPase activity, the goal of this study was to correlate the effects of LPS on intestinal Na^+,K^+-ATPase activity and BT in iNOS knockout mice (iNOS-/-). **Methods:** iNOS-/- mice and their wild type litter mates (iNOS+/+) received LPS(10mg/kg) intraperitoneally. Two, six, and 18 hours later, ileal Na^+,K^+-ATPase activity was measured. BT to the mesenteric lymph nodes(LMN) was determined 18 hours after LPS challenge. **Results:** Na^+,K^+-ATPase activity was decreased in both iNOS+/+ and iNOS-/- mice 2 hours after receiving LPS compared to control mice receiving saline (Table). 6 hours after LPS challenge, Na^+,K^+-ATPase activity in iNOS+/+ mice was lower than iNOS-/- and by 18 hours after LPS, Na^+,K^+-ATPase activity had returned to normal in iNOS-/- mice receiving LPS. The incidence of BT to MLN after LPS challenge was significantly higher in the iNOS+/+ mice than the other groups. Na^+,K^+-ATPase activities in the mice in which BT did not occur were significantly higher than in mice in which BT occurred (17±5 vs. 9±4, p<0.05).

Group	Na^+,K^+-ATPase activity (μmol/h/mg protein)			BT(%)
	2 hours	6 hours	18 hours	18 hours
iNOS+/+ LPS	10±2[a]	5±1[a,b]	11±4[a]	87.5[c]
iNOS+/+ Saline	20±3	24±6	25±8	0
iNOS-/- LPS	11±2[a]	13±4[a]	16±6	12.5
iNOS-/- Saline	20±3	21±6	23±7	0

N=6-8/group; Data are expressed as mean±SD; [a] p<0.01 vs. Saline. [b] p<0.01 vs. iNOS-/- LPS group. [c] p<0.01 vs. other groups.

Conclusion: LPS-induced BT was associated with prolonged decreases in ileal Na^+,K^+-ATPase activity. These results suggest that inhibition of this enzyme might be involved in LPS-induced gut barrier dysfunction. In addition, since iNOS knockout mice showed a faster recovery of Na^+,K^+-ATPase activity after LPS challenge , it appears that LPS-induced NO may be associated with prolongation of LPS-induced inhibition of Na^+,K^+-ATPase activity.

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EXPRESSION OF DIFFERENT PDH KINASE ISOENZYMES IN SKELETAL MUSCLE DURING SEPSIS. T. C. Vary, and G. Deiter*, Dept. Cellular and Molecular Physiology, Penn State University College of Medicine, Hershey, PA. 17033 USA

Hyperlactatemia is a frequent complication of sepsis. Increased lactate production by skeletal muscle elevates plasma lactate concentrations. Inhibition of the PDH complex contributes to the accelerated release of lactate from skeletal muscle. The inhibition of PDH activity resides in a stimulation of PDH kinase (PDHK) during sepsis. Recent evidence indicates the existence of multiple isoenzymes of PDK. In skeletal muscle, the PDK2 and PDK4 isoenzymes predominate. We investigated whether sepsis would have specific effects on the expression of PDK isoenzymes mRNA in skeletal muscle by Northern blot analysis. We also examined the time course of changes in the expression of the isozymes following induction of sepsis. PDK2 and PDK4 Northern blots were normalized by GPDH mRNA. The relative amount of PDK2 and PDK4 mRNA are shown in the Table below:

	3 DAYS		5 DAYS	
	Control	Sepsis	Control	Sepsis
PDK2	98±2	113±21	88±5	**171±24***
PDK4	12±4	**50±9***	60±10	45±12

Values shown are arbitrary units of means ±SE for 3-7 animals in each group. *P<0.05 vs Control at same time.

The results indicate that isoenzymes of PDK are differentially expressed during the course of the septic episode. (Supported by GM-50919)

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CARDIAC RESPONSE TO NITRIC OXIDE SYNTHASE INHIBITION USING AMINOGUANIDINE IN A RAT MODEL OF ENDOTOXEMIA.
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This study evaluates the effect of aminoguanidine, a preferential inhibitor of inducible nitric oxide synthase (iNOS), on the prevention of cardiac depression in acute endotoxemia. Cardiac performance was evaluated after 4 hr of exposure to endotoxin. Animals were randomly selected to receive by i.p. injection one of 4 treatments (n=5 per treatment); Saline, LPS (lipopolysaccharide, E.Coli, 4mg/kg), AG (aminoguanidine 100 mg/kg), and LPS + AG at various times. AG and saline treatments were administered 30 min before LPS and at 1 and 3 hr after LPS injection. Hearts were perfused using the Langendorff preparation and a balloon tipped catheter was placed in the left ventricle to measure left ventricular developed pressure (LVDP). Myocyte contractile function was assessed with electrical field stimulation and video microscopy. Tissue was immunostained for the expression of iNOS and for nitrotyrosine, a byproduct of protein nitration by peroxynitrite. Perfused hearts from LPS-treated rats exhibited a 57% decrease (P=.0001) in LVDP compared to saline-treated animals. No improvement in ventricular function was observed with the administration of AG. Similarly, cardiac myocytes prepared from LPS-treated animals demonstrated a significant (P<.05) reduction in percent and velocity of shortening and this effect was unaltered with the same dose of AG. Aminoguanidine administration significantly (P=.0003) reduced serum nitrite/nitrate levels in endotoxemic rats to control levels. Localized expression of iNOS in the myocardium was lessened with AG treatment and was not associated with peroxynitrite formation in this model of endotoxemia.The results indicate that AG given i.p. before and after endotoxin (at a concentration sufficient to decrease nitric oxide production) did not reduce cardiac depression. We conclude that selective inhibition of iNOS and reduction of nitric oxide production do not prevent cardiac dysfunction in an acute model of endotoxic shock.

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HIGH MOBILITY GROUP-1 (HMG-1) PROTEIN IS A MEDIATOR OF LETHAL ENDOTOXEMIA.
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Lethal endotoxemia stimulates the release of macrophage-derived cytokines (e.g. TNF-α and IL-1β) that mediate shock. Death, however, frequently occurs several days later when serum TNF-α and IL-1β levels have returned to basal values. To discover previously unrecognized macrophage-derived mediators of endotoxin toxicity released late in endotoxemia, conditioned medium of LPS-stimulated macrophage (RAW 264.7) cell cultures was screened by SDS-PAGE for proteins appearing 8 hours or more after LPS stimulation. An LPS-induced 30 kDa protein was identified by N-terminal amino acid sequence as HMG-1 protein. HMG-1 was released from murine macrophage and human peripheral blood mononuclear cell cultures following stimulation with LPS, TNF-α, or IL-1β. Although undetectable in serum of normal mice, HMG-1 levels increased significantly at least 8 hours after the onset of endotoxemia, and remained at plateau levels (up to 350 ng/ml) for 16-32 hours. Passive immunization of mice with anti-rHMG-1 antibodies decreased the lethality from 100% in control group (treated with LD_{100} dose of LPS and pre-immune serum), to less than 30% in experimental group (treated with LD_{100} dose LPS and anti-rHMG-1 serum). Co-administration of purified rHMG-1 protein synergistically increased LPS lethality from 0% in group treated with LPS alone (3.25 mg/kg), to more than 80% in group treated with LPS (3.25 mg/kg) plus rHMG-1 (50 μg/mouse). Serum HMG-1 levels in patients with lethal septicemia were significantly increased (83.7 ± 27.5 ng/ml) compared to either normal subjects (non-detectable) or patients with non-lethal sepsis (25.2 ± 17.8 ng/ml). Thus, HMG-1 is a previously unrecognized mediator of endotoxin lethality, which can be targeted as future therapeutics.

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ADRENAL INSUFFICIENCY DURING THE LATE STAGE OF POLYMICROBIAL SEPSIS. P. Wang, D.J. Koo*, I.H. Chaudry. Brown University School of Medicine and Rhode Island Hospital, Providence, RI 02903.

Although studies have indicated that adrenal insufficiency occurs following severe hemorrhagic shock, it remains controversial whether adrenal function is depressed during polymicrobial sepsis. To study this, male rats (~300g) were subjected to sepsis by cecal ligation and puncture (CLP) or sham operation followed by the administration of normal saline solution. Systemic blood samples were taken at 20 h after CLP or sham operation to measure plasma levels of corticosterone (ng/mL) and ACTH (pg/mL) as well as adrenal contents of corticosterone (ng/mg tissue). Additional groups of animals

were utilized to examine ACTH-stimulated (100 μg/rat) corticosterone release. The data (mean ± SE, n=5-8/group) are as follows:

	Sham	CLP
Plasma ACTH	57 ± 3	100 ± 9*
Plasma corticosterone	196 ± 29	191 ± 41
Adrenal corticosterone	65 ± 5	38 ± 4*
ACTH-stimulated corticosterone increase	260 ± 7	122 ± 6*

(Student's t-test; * $p < 0.05$ vs. Sham)

The results indicate that despite a marked increase in plasma levels of ACTH at 20 h after the onset of sepsis, plasma corticosterone levels were similar to those in sham-operated animals. In addition, adrenal contents of corticosterone decreased in septic animals. Moreover, the net increase in corticosterone induced by ACTH was decreased by 53% at 20 h after CLP. These findings suggest that despite high plasma levels of endogenous ACTH as well as stimulation by exogenous ACTH, adrenal dysfunction as indicated by reduced plasma corticosterone release and decreased adrenal contents of corticosterone occurs during the late stage of polymicrobial sepsis. These results lead us to conclude that the recognition of adrenal insufficiency and interventions to improve adrenal responsiveness may be beneficial in improving the outcome during sepsis (Supported by NIH GM 53008).

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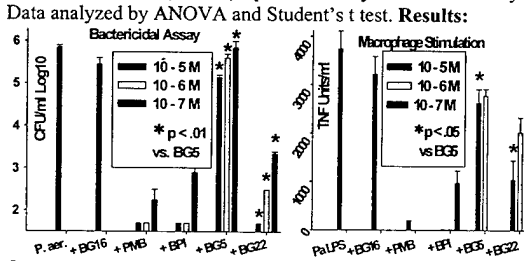
LOCALIZATION OF BACTERICIDAL AND ENDOTOXIN NEUTRALIZING ACTIVITY TO A COMMON DETERMINANT OF BACTERICIDAL/ PERMEABILITY-INCREASING PROTEIN (BPI). C. Weiss*, K. Wasiluk*, T. Kellogg*, D. Dunn. Univ. of Minnesota, Dept. of Surgery, Minneapolis, MN 55455.

BPI is a 55 kD neutrophil-derived mammalian protein that: **1)** binds the toxic lipid A component of gram-negative bacterial lipopolysaccharide (endotoxin, LPS), and **2)** is bactericidal. However, confusion exists regarding whether these activities reside within the same or disparate molecular regions of BPI. This knowledge is critical in the development of synthetic anti-LPS peptides. BPI's bactericidal activity has been localized to amino acids (a.a.) #90-99, but a peptide derived from this region (BG5) was not bactericidal. We hypothesized that a larger synthetic peptide containing BPI a.a. #82-108 (BG22) would **1)** neutralize LPS, and **2)** retain bactericidal activity.

Methods: Bactericidal activity: *P. aeruginosa* incubated with either BPI, BG5, BG22, positive control polymyxin B (PMB), or BG16 (an irrelevant peptide) and colonies enumerated. LPS-neutralization: *P. aeruginosa* LPS incubated with either BPI, BG5, BG22. Inhibition of LPS-induced secretion of tumor necrosis factor-α (TNF-α) quantitated by WEHI bioassay. Data analyzed by ANOVA and Student's t test. **Results:**



Conclusion: BG22 (a.a. #82-108) is bactericidal and neutralizes *P. aeruginosa* LPS in contrast to BG5 (a.a. #90-99). These data represent the first indication that both activities reside within the same molecular domain of BPI.

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ADENOVIRAL VECTOR TRANSFECTION INTO THE PULMONARY EPITHELIUM AFTER CECAL LIGATION AND PUNCTURE (CLP) IN RATS . Yoram G. Weiss*, Arthur Bouwman*, Beth Gehan*, John Tazelaar* and Clifford S. Deutschman. Department of Anesthesia, and the Institute for Human Gene Therapy, University of Pennsylvania, Philadelphia, PA 19104.

Adenoviral-targeted gene delivery to the respiratory epithelium is a potential method to transiently augment specific protein production. The pathophysiology of sepsis-induced Acute Respiratory Distress Syndrome (ARDS) may involve changes in protein expression. Therefore, we examined the temporal uptake of adenoviral vectors after CLP, hypothesizing that an increase will occur in this model of ARDS. All studies conformed to NIH guidelines for the treatment of laboratory animals. CLP was produced as previously described. Sham operated animals served as controls. $3x10^{12}$ or $3x10^{11}$ viruses containing a vector to express Green Fluorescent Protein (GFP), a marker protein, were delivered via a tracheal catheter. Rats were sacrificed at 24, 48 and 72 hours. Lungs were removed en-bloc, inflation-fixed and sectioned. H&E histopathology and GFP were identified using light and fluorescence microscopy respectively. H&E staining demonstrated lung pathology consistent with ARDS. High levels of adenoviral-mediated GFP expression were observed in small airways and surrounding alveoli. GFP expression was most prominent in mildly to moderately diseased areas but not in severely diseased regions. Uptake was more pronounced than in control animals. The lower dose resulted in comparable uptake with less lymphocytic (i.e., viral mediated) infiltration. We conclude that adenoviral-mediated gene transfer is enhanced by CLP and is highly efficient in this ARDS model, perhaps because airway epithelial cells over-express an adenoviral receptor during lung injury.

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SUPPRESSING EARLY ACTIVATION OF TISSUE NFκB AND NF-IL6 POSITIVELY CORRELATES WITH IMPROVED SURVIVAL IN POLYMICROBIAL SEPSIS. D. Williams*, T. Ha*, C. Li*, J. Laffan*, D. Ferguson*, J. Kalbfleisch* P. Kougias* and W. Browder* (Spon: J. Cook). Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37601.

Recent data implicate the activation of nuclear factor-kappaB (NFκB) and nuclear factor interleukin 6 (NF-IL6) as important initial steps in the pathophysiology of adult respiratory distress syndrome (ARDS) and sepsis syndrome. This study evaluated the effect of two immunomodulating polysaccharides, glucan phosphate and scleroglucan, on transcription factor activation, pro-inflammatory cytokine mRNA expression and mortality in a murine model of sepsis/shock induced by cecal ligation and puncture (CLP). CLP increased ($p<0.05$) liver and lung NFκB and NF-IL6 nuclear binding activity as well as TNFα and IL-6 mRNA levels at 3 hrs, when compared to the sham operated group. ICR/HSD mice were treated with glucans (50 mg/kg) 1 hr prior to or 15 min following CLP. Sham operated (laparotomy only) mice served as the surgery/anesthesia control. Liver and lung tissue were harvested at 3 hr and mortality trends were observed for 20 days. Prophylaxis or therapy with glucans decreased ($p<0.05$) liver and lung NFκB and NF-IL6 binding activity (58 to 77%) as well as TNFα and IL-6 mRNA levels. Prophylaxis with glucan phosphate or scleroglucan increased ($p<0.001$) long-term survival (20% CLP vs 65% glucan phosphate, 75% scleroglucan). Therapy with glucan also increased ($p<0.05$)

long-term survival (20% vs 65%). We observed that pre- or post-treatment with (1→3)-β-D-glucan biological response modifiers decreased tissue transcription factor nuclear binding activity and pro-inflammatory cytokine mRNA in liver and lung of septic mice. Of greater significance, the data suggest that blunting early transcription factor activation and cytokine message expression correlates with improved outcome in polymicrobial sepsis as denoted by increased long-term survival.

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Na^+,K^+-ATPASE ACTIVITY IS INHIBITED IN CULTURED INTESTINAL EPITHELIAL CELLS BY ENDOTOXIN AND NITRIC OXIDE

D. Xu, Y. Suzuki*, Q. Lu*, E. Deitch, UMDNJ-New Jersey Med. Sch., Newark, NJ, 07103

Introduction: Na^+,K^+-ATPase is an important enzyme which serves vital functions in various mammalian tissues, including the intestine. We previously have documented that endotoxin (LPS) and nitric oxide (NO) can induce entrocyte injury in vitro. To test whether Na^+,K^+-ATPase plays a role in LPS- or NO-induced enterocyte injury, we examined Na^+,K^+-ATPase activity in rat intestinal epithelial cells (IEC-6) after exposure to LPS or NO. **Methods:** IEC-6 cells were incubated with various concentrations of LPS, the NO donor, S-Nitroso-N-acetylpenicillamine (SNAP), or medium for designed incubation periods. Na^+,K^+-ATPase activity was assayed as the ouabain-sensitive hydrolysis of ATP. The amount of inorganic phosphate liberated by the hydrolysis of ATP was measured spectrophotometrically. **Results:** Na^+,K^+-ATPase activity was 21±3 μmol/h/mg protein in IEC-6 cells incubated with medium. When the IEC-6 cells were exposed to LPS at concentration of 25μg/ml for one, two, six or 24 hours, Na^+,K^+-ATPase activity was significantly decreased to 3-4μmol/h/mg protein at all incubation periods ($p<0.01$ vs. control). When the cells were exposed to LPS at 0.25, 1.0, 2.5, or 25μg/ml for one hour, Na^+,K^+-ATPase activities decreased in a dose-dependant manner (16, 9, 6 and 3μmol/h/mg protein respectively). Incubation with SNAP at 0, 0.01, 0.1, 1 or 2 mM for 6 hours also inhibited Na^+,K^+-ATPase activity in a dose-dependent manner. At as low a SNAP dose as 0.1mM significant inhibition of Na^+,K^+-ATPase activity was observed. (15±2 vs. 21±3μmol/h/mg protein, $p<0.05$ vs. medium). **Conclusion:** These results showing that LPS or the NO donor, SNAP, inhibit Na^+,K^+-ATPase activity in IEC-6 cells suggests that inhibition of Na^+,K^+-ATPase activity by LPS or NO may be involved in LPS- or NO-induced intestinal mucosal injury.

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DIFFERENTIAL ALTERATIONS IN SYSTEMIC AND REGIONAL OXYGEN DELIVERY AND CONSUMPTION DURING THE EARLY AND LATE STAGES OF SEPSIS.

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Although polymicrobial sepsis is characterized by an early, hyperdynamic phase followed by a late, hypodynamic phase, the differential alterations in systemic and regional oxygen delivery (DO_2) and consumption (VO_2) at different stages of sepsis remain unknown. To determine this, male adult rats (n=7/group) were subjected to polymicrobial sepsis by cecal ligation and puncture (CLP) followed by fluid resuscitation. At 5 h (i.e., the early stage of sepsis) or 20 h (the late stage) after CLP, cardiac output (CO) and organ blood flow were determined by radioactive microspheres. Systemic and regional DO_2 and VO_2 were calculated and plasma levels of lactate were also measured. The results indicate that CO and blood flow in the liver, small intestine, and kidneys increased by 29.3-34.5% ($P<.05$) at 5 h and decreased by 40.9-68.2% ($P<.05$) at 20 h after CLP. Systemic DO_2 and VO_2 increased by 35.9% and 43.1% ($P<.05$), respectively, at 5 h after CLP. In contrast, systemic DO_2 but not VO_2 decreased by 41.2% ($P<.05$) at 20 h after CLP. At 5 h after CLP, DO_2 in the small intestine and kidneys, but not in the liver, increased significantly. At 20 h after CLP, however, DO_2 in the tested organs decreased by 43.9-69.7% ($P<.05$). Organ VO_2 increased by 36.8-78.9% ($P<.05$) in all the tested organs at 5 h after CLP, but decreased significantly in the liver and gut at 20 h after the onset of sepsis. In addition, plasma levels of lactate increased by 138.5% ($P<.05$) at the late stage of sepsis, indicating tissue hypoxia. In line with the changes in CO and organ perfusion, systemic and organ DO_2 increased during the early stage and decreased during the late stage of sepsis. Since hepatic and intestinal VO_2, but not renal VO_2 decreased significantly at 20 h after CLP, the liver and gut appear to be more vulnerable to the hypoxic insult during the late stage of polymicrobial sepsis (Supported by NIH GM 53008).

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ANTI-IFN-γ ANTIBODY AMELIORATES PULMONARY SUPEROXIDE PRODUCTION AFTER CECAL LIGATION AND PUNCTURE IN RATS.

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Interferon-gamma (IFN-γ) has been implicated in the mortality of animal models of endotoxemia. However, the specific role of IFN-γ in the development of organ inflammation in a model of polymicrobial sepsis has not been elucidated. We report here that IFN-γ plays a role in lung inflammation after cecal ligation and puncture (CLP). Lung tissue was removed 5h after CLP or from sham controls. The mRNA expression (by RT-PCR) of IFN-γ was increased in lung homogenates of CLP rats compared to sham controls. Using immunohistochemistry, we show here, for the first time, an increase of IFN-γ staining cells in the lung following CLP. Only very small amounts of positive staining for IFN-γ were detected in lungs of sham controls under identical conditions. The presence of IFN-γ in lung 5h following CLP, correlated with two-fold increases in lung superoxide generation and MPO activity (index of neutrophil sequestration). Plasma and lung nitrite levels (breakdown product of nitric oxide) were also increased significantly in CLP rats. IFN-γ antibody (1.2 mg/kg, i.v.) administered immediately after CLP, ameliorated lung superoxide levels to similar levels as sham controls without affecting MPO activity. Lung and plasma nitrite levels were not affected by administration of IFN-γ antibody. These results suggest that IFN-γ contributes to lung inflammation 5 h following CLP through the activation of superoxide producing cells.

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ERYTHROCYTE INJURY BY ENDOTOXIN AND PROTECTIVE EFFECT OF ANISODAMINE IN DOGS

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Recent studies have indicated that anisodamine proved beneficial effect for the survival of animals with endotoxic shock. However its mechanism is unknown. The present study was

undertaken to determine whether anisodamine protects erythrocytes from injury induced by endotoxin. 16 dogs suffered from endotoxemia were divided into two groups at random. The animals in test group received anisodamine(7mg/Kg) and in the control group same volume of normal saline. Arterial blood sample were drawn before (T0) and 10 hours(T10) after endotoxin injection. Plasma was for detection of free hemoglobin (PFHb, Crosby's method) and erythrocytes for measurements of intracellular Na^+ ($[Na^+]_i$) and K^+($[K^+]_i$) (spectrophotometric method). The results showed that when compared to T0, at T10 in the control group PFHb increased 77.2 ±9.7%, $[Na^+]_i$ also increased 22.4±1.9%. but $[K^+]_i$ decreased 13.0 ±1.5%; in the anisodamine group the changes of the indexes were markedly attenuated, PFHb increased 30.4± 6. 8%, $[Na^+]_i$ increased 14.5±2.0% and$[K^+]_i$ decreased 7.6±1.6%. The facts suggest that erythrocytes are damaged during endotoxemia and anisodamine has protective effect on the pathological process.

To test it in vitro,. blood samples were drawn and added with endotoxin + normal saline/buffer and endotoxin + anisodamine /buffer, then cultured at 37℃ for 2 hours. Supernatants were separated for detections of PFHb and malonyldialdehyde (MDA). In contrast to the control group,. PFHb and MDA in anisodamine group were remarkably lower. Therefore it would appear that the protective effect of anisodamine might be associated with the inhibition of lipid peroxidation.

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THE SMALL INTESTINE IS A MAJOR SOURCE OF ADRENOMEDULLIN RELEASE DURING POLYMICROBIAL SEPSIS. M. Zhou*, I.H. Chaudry and P. Wang. Brown University School of Medicine and Rhode Island Hospital, Providence, RI 02903.

Previous studies have indicated that plasma levels of adrenomedullin (ADM), a novel vasodilatory peptide, increased significantly during the early and late stages of sepsis. Although ADM gene expression is upregulated in the small intestine during sepsis, it remains unknown whether the gut is a major source of the ADM release under such conditions. To determine this, male rats (275-325g) were subjected to sepsis by cecal ligation and puncture (CLP) followed by fluid resuscitation. Systemic and portal blood were taken simultaneously at 10 or 20 h after CLP or sham-operation. Intestinal samples were also harvested at the above time points. Levels of ADM were measured by RIA. The localization of ADM in the small intestine was examined using an immunohistochemistry technique. The plasma and tissue levels of ADM (pg/ml plasma or pg/mg protein in tissue; n=6-8/group; mean ± SE) were as follows:

	10 h		20 h	
	Sham	CLP	Sham	CLP
Systemic	200±14	582±58*	207±18	535±25*
Portal	216±22	826±92*#	233±20	744±60*#
Tissue	7.5±0.5	20.9±2.4*	8.0±0.8	13.7±1.1*

(Student's *t*-test: *P<0.05 vs. Sham; #P<0.05 vs. Systemic)

The results indicate that ADM levels in the portal blood were significantly higher than in systemic blood at 10 and 20 h after CLP. ADM levels in the small intestine were also significantly increased at both time points. Immunohistochemistry showed that ADM was localized in the mucosa and submucosa of the small intestine and the ADM immunostaining was significantly increased at 10 and 20 h after CLP. Thus, the small intestine appears to be a major source of ADM release during polymicrobial sepsis. (Supported by NIH GM 57468).

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eNOS TRANSLOCATION FROM CAVEOLAE: AN IMPORTANT MECHANISM IN SEPSIS-INDUCED ENDOTHELIAL CELL DYSFUNCTION. W.A. Arden, G. Gellin*, E.J. Smart*. **University of Kentucky Medical Center, Lexington, KY 40536-0084.**

Failure of endothelial cell (EC) nitric oxide synthase (eNOS) signaling is a documented component of sepsis-induced microvascular dysfunction. In healthy ECs, eNOS resides in specific plasma membrane domains known as caveolae. We examined the hypothesis that lipopolysaccharide (LPS) and select cytokines induce translocation of eNOS from caveolae with concomitant loss of functional activity. Human microvascular ECs were exposed to E.coli LPS (1ug/ml), interleukin-1β (IL-1:3 ng/ml), tumor necrosis factor-α (TNF:10 ng/ml) and interferon-γ (IFN:100 u/ml), alone and in combination, or vehicle (control). After 24 hours, cells were harvested and the following highly purified membrane fractions prepared: internal (IM), plasma (PM) and caveolar (CM). Fractions were then immunoblotted for eNOS specific activity. Localization of eNOS to the CM fraction in control cells was confirmed. Treatment with LPS, IL-1 or the combination TNF/IFN had no effect on eNOS localization. The combinations LPS/IL-1 and LPS/TNF/IFN caused complete depletion of eNOS from CM. The study was repeated and immunoblots performed using cell equivalent loading. This technique confirmed depletion of eNOS activity from CM and revealed translocation to the IM fraction. In a third study, eNOS activity was quantitated in the above subcellular fractions by measuring the conversion of [^{3}H]arginine to[^{3}H]citrulline. LPS/IL-1 and LPS/TNF/IFN treatments increased eNOS activity in the IM fraction from 6.4±1.1 (control) to 8.9±2.4 and 7.3±1.6 pmol/ug protein/min respectively. Activity in the CM fraction was no longer detectable (control: 53.2±3.1). These data strongly suggest that while LPS and cytokines alone have little effect on eNOS localization, the combination of LPS and cytokines potently stimulates the translocation of eNOS from its functional caveolar domain to alternate internal membrane sites. Such translocation may be a fundamental mechanism in sepsis-induced failure of eNOS signaling.

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REGULATION OF FREE RADICAL RELEASE, CHEMOKINE PRODUCTION, ADHESION MOLECULE EXPRESSION AND LIVER INJURY DURING ALCOHOL WITHDRAWAL AND REPERFUSION AFTER HEPATIC ISCHEMIA. AP Bautista, EA Arruebarrena, J Carnal, A Shoichet, A Dobrescu, S Munshi. Physiology Dept., LSU Medical Center, New Orleans, LA 70112.

Reactive oxygen species (ROS), chemokines and adhesion molecules are implicated in the pathogenesis of liver injury during reperfusion after hepatic ischemia (Jaeschke et al., *Free Radic. Res. Commun. 15*:277-284) and alcohol intoxication (Bautista, *Hepatol. 25*:335-342). Enhanced ROS production and hepatic dysfunction are also observed during withdrawal after an acute alcohol binge (Bautista & Spitzer *Alcohol. Clin. Exp. Res.20;*502-509). Male Sprague-Dawley rats were given an intravenous infusion of ethanol to maintain blood ethanol level at 150-175 mg/dl. After 12 hr, ethanol was withdrawn and replaced by saline for another 6 hr of infusion, and were subjected to partial hepatic ischemia for 45 min followed by reperfusion for 1 to 24 hr. Serum ALT level was higher at 3 hr of reperfusion in alcohol withdrawal plus I/R group, compared with the parallel control. ROS production by isolated Kupffer cells at 3 and 24 hr after reperfusion was higher in alcohol plus I/R-rats than those of saline plus I/R group. After 24 hr of reperfusion, serum ALT in the alcohol-treated rats was decreased by 90% compared with 10% in the non-alcohol-treated group. Serum α (MIP-2) & β-chemokines (MIP-1α , MCP-1, RANTES) levels were lower in rats subjected to alcohol withdrawal and I/R compared with the I/R group previously treated with saline at 3 hr. After 24 hr, there was no significant difference in serum chemokine levels

between these two treatment groups. Acute alcohol binge followed by withdrawal was associated with 50% reduction in number of CD11b/c positive leukocytes in the liver at 24 hr of reperfusion, compared with saline I/R-treated group. These data suggest that acute alcohol exposure followed by withdrawal may elicit tissue injury during the early phase of reperfusion after hepatic ischemia, but may provide protection at a later phase of reperfusion. (Supported by NIH-AA 8846, 10466).

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CYTOKINES TNF/IFN AND NEUROPEPTIDE CGRP SUPPRESS SMOOTH MUSCLE ARGINASE ACTIVITY

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We have previously demonstrated that calcitonin gene related peptide (CGRP), released during endotoxemia and acute inflammation, markedly enhances lipopolysaccharide (LPS)- and cytokine-induced nitric oxide synthase (iNOS) activity and NO production in vascular smooth muscle (VSM). iNOS competes with several other enzymes, including arginase, for access to the substrate L-arginine. We hypothesized that these same agents would suppress VSM arginase activity, increasing available L-arginine during acute inflammatory stress. Rat aortic VSM cells were grown to confluence and treated with E.coli LPS (1ug/ml), interleukin 1-β (IL-1:3 ng/ml), tumor necrosis factor-α (TNF:10 ng/ml), interferon-γ (IFN:100U/ml), alone or in combination, and vehicle (control). These treatments were repeated in the presence of CGRP (10^{-8}M) or 8-br-cAMP (3mM). Cells were removed after 24 hours and assayed for total arginase activity by measuring conversion of L-arginine to L-ornithine. LPS alone mildly stimulated VSM arginase activity, while IL-1 alone and in combination with LPS had little effect. The combinations TNF/IFN and LPS/TNF/IFN markedly suppressed VSM arginase activity (98.6±5 and 67±2.5 respectively vs. 153±5.5 control: nmol/mg protein/min) ($p<0.05$). Interestingly, CGRP alone slightly increased basal VSM arginase activity (165±2.9), but enhanced TNF/IFN- and LPS/TNF/IFN-induced suppression of activity (59.8±3 and 43.5±3.8 respectively) ($p<0.05$). The same pattern was observed with 8-br-cAMP. These data strongly suggest that the neuropeptide CGRP, in combination with TNF/IFN, markedly suppresses VSM arginase activity, potentially increasing L-arginine availability for iNOS and NO production during acute inflammatory stress within the vascular wall. The mechanism of this enhancement is compatible with VSM generation of cAMP.

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DOES NITRIC OXIDE SYNTHASE INHIBITION INCREASE TESTOSTERONE VIA STEROIDOGENIC ACUTE REGULATORY PROTEIN (StAR) IN SEPSIS?

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In the present study we hypothesized that blockade of nitric oxide synthase (NOS) by N^G-nitro-L-arginine methyl-ester (L-NAME) would affect Leydig cell steroidogenesis and increase the production of testosterone *in vivo* and *in vitro*. Male Sprague-Dawley rats (350-450 g), were randomized to septic and non-septic groups. Sepsis was induced with an ip injection of a cecal slurry (200 mg/kg in 5 ml 5% dextrose in water (D_5W); ip) in rats, while non-septic rats received only sterile D_5W. Testes from septic and sham-septic rats were collected and Leydig cells were purified using a modified percoll gradient method. Leydig cells were treated with L-NAME (30 μM) for thirty minutes. Supernatant was collected for estimation of testosterone and cells were collected for Western blot analysis. In another group, non-septic and septic rats were infused with L-NAME (0.5 mg/kg·min) for ten min and the serum concentration of testosterone was determined using radioimmunoassay. As previously reported, at 24 hours heart rate and NO byproducts (NOx) levels were significantly increased while the serum concentration of testosterone was significantly decreased in septic rats. Infusion of L-NAME significantly reduced serum NOx levels in septic rats. The infusion of L-NAME significantly increased serum concentration of testosterone in non-septic and septic rats. Sepsis produced a significant decrease in the levels of StAR in Leydig cells. Treatment with L-NAME increased the concentration of testosterone in the supernatant of Leydig cells obtained from septic rats. It is concluded that elevated NO during sepsis decreases serum testosterone levels which is correlated with a decrease in StAR.

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Beneficial effects of MN(III)tetrakis (4-Benzoic acid) Porphyrin (MNTBAP), a superoxide dismutase mimetic, in zymosan-induced non-septic shock

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The therapeutic efficacy of Mn(III)tetrakis (4-benzoic acid) porphyrin (MnTBAP), a novel superoxide dismutase mimetic which scavengers peroxynitrite, was investigated in rats subjected to non-septic shock induced by peritoneal injection of zymosan. Our data show that MnTBAP (given at 3 and 10 mg/kg intraperitoneally, 1 and 6 h after zymosan injection) significantly reduce in dose dependent manner the development of peritonitis (peritoneal exudation, high nitrate/nitrite and peroxynitrite plasma levels, leukocyte infiltration and histological examination). Furthermore, our data suggest that there is a reduction in the lung, small intestine and liver myeloperoxidase (MPO) activity and lipid peroxidation activity from MnTBAP-treated rats. MnTBAP also reduced the appearance of nitrotyrosine immunoreactivity in the inflamed tissues. Furthermore, a significant reduction of suppression of mitochondrial respiration, DNA strand breakage and reduction of cellular levels of NAD^+ was observed in ex vivo macrophages harvested from the peritoneal cavity of zymosan-traeted rat.

In vivo treatment with MnTBAP significantly reduced in a dose-dependent manner peroxynitrite formation and prevented the appearance of DNA damage, the decrease in mitochondrial respiration and the loss of cellular levels of NAD^+. In conclusion our results showed that MnTBAP was effective in preventing the development of zymosan-induced non-septic shock.

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EFFECTS OF ENDOTOXEMIA ON SUSTAINED ENDOTHELIUM-DEPENDENT RELAXATION, UNDERLYING MEDIATORS, AND AGONIST-STIMULATED CYTOSOLIC Ca^{2+} RESPONSES

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Agonist-mediated endothelium-dependent relaxation (EDR) involves time-dependent release of multiple mediators including nitric oxide (NO), vasoactive prostanoids (PG), and

endothelium-derived hyperpolarizing factor (EDHF). In the current study we evaluated effects of endotoxemia on EDR and time-dependent roles of underlying mediators, during sustained (30 min) exposure to selected endothelium-dependent agonists. Since synthesis of mediators often occurs via Ca^{2+}-dependent enzymes, we also evaluated effects of endotoxemia on cytosolic Ca^{2+} responses using freshly dispersed aortic endothelial cells. Aortae and mesenteric (MES) near-resistance arteries (~150 μm) were isolated from guinea pigs 16 hrs post treatment with sterile saline (CON) or purified E coli endotoxin lipopoly-saccharide (LPS). In MES preconstricted with endothelin-1 (ET-1), EDR to acetylcholine (ACh; 0.3 uM) @ 2 min was not different between LPS and CON (46% vs 49%, respectively); however, from 2 –30 min, EDR of LPS MES progressively waned to 22% ; EDR increased in CON MES to 60% @ 30 min. EDR @ 30 min was absent in LPS MES after inhibition of NO synthase (300 uM LNMMA). ADP (10 uM) produced a slow, sustained relaxation in CON but not LPS MES; LNMMA completely blocked this response. In aortae, both initial and sustained EDR to ACh were markedly impaired by LPS treatment. In the absence of inhibitors, EDR to ADP (10 uM) in aortae was not impaired by LPS; however, after inhibition of cyclooxygenase and EDHF, impaired NO-dependent EDR was evident. Freshly dispersed endothelial cells from LPS-treated animals showed markedly inhibited ACh and ADP-stimulated cytosolic Ca^{2+} responses, suggesting that decreased agonist-stimulated Ca^{2+} responses and reduced Ca^{2+}-dependent NOS activity are mechanisms underlying decreased NO synthesis and impaired EDR in endotoxic shock states. Furthermore, NO-dependent mechanisms play a major role in sustained EDR during prolonged exposure to agonists. Support: AHA-National

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DIFFERENTIAL EFFECTS OF ENDOTOXEMIA ON ENDOTHELIUM-MEDIATED RELAXATION (EDR) AND ENDOTHELIN CONTRACTIONS OF CORONARY, MESENTERIC AND PULMONARY ARTERIES

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We previously documented impaired EDR and decreased agonist-stimulated nitric oxide (NO) synthesis of aortae isolated during guinea pig endotoxemia. In the current studies, we compared EDR and contractile responses to endothelin-1 (ET-1) in pulmonary branch arteries, coronary and mesenteric microvessels (~150 um), isolated from the same model of endotoxemia. Specialized microvessel myographs and isometric techniques were used to compare responses of vessels isolated from endotoxin-treated (16 hrs post ip injection of 4 mg/kg purified E. coli endotoxin [LPS]) and control (sterile saline-treated) animals. Mesenteric and coronary microvessels isolated from LPS-treated animals demonstrated significantly impaired EDR to acetylcholine (ACh; 10^{-8}– 10^{-4} M), similar to impaired EDR observed in aortae. In contrast, pulmonary branch arteries isolated from LPS-treated animals exhibited significantly enhanced EDR to both ACh and ADP (10^{-9}-10^{-5} M) ($p<.05$). Furthermore, LPS-induced enhanced EDR of pulmonary arteries was reversed in the presence of cyclooxygenase (COX) inhibition (indomethacin), but not NO synthase inhibition (L-NMMA), suggesting selective upregulation of a COX-dependent prostanoid vasodilator in pulmonary, but not mesenteric or coronary arteries. Contractile responses to ET-1 were significantly enhanced ($p<.01$) in mesenteric but not in coronary microvessels, aortic or pulmonary arteries. Contractile responses to prostaglandin $F_{2\alpha}$ were decreased in all vessels isolated from LPS-treated animals. Our combined data confirm differential effects of *in vivo* LPS on EDR and underlying endothelium-dependent mechanisms (NO, prostanoids) in these vascular beds. Importantly, impaired EDR in combination with increased ET-1 contractions observed in mesenteric microvasculature may contribute to abnormal blood flow regulation during endotoxic/shock states. Support: AHA-National

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EXERCISE TRAINING ENHANCES ENDOTHELIUM-DEPENDENT RELAXATION OF PORCINE CORONARY ARTERIES DISTAL TO CHRONIC CORONARY OCCLUSION.

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Chronic myocardial ischemia and collateral-dependent perfusion have been reported by several laboratories to impair endothelium-dependent relaxation responses (EDR). In the current studies, we investigated potential beneficial effects of exercise training on EDR of coronary arteries isolated from a porcine model of chronic, complete coronary occlusion. Two months after surgical placement of an ameroid occluder around the proximal left circumflex coronary artery (LCX), animals were either pen restricted (SED) or exercise trained on a motor driven treadmill (EX) for 16 weeks. Conduit (>1.0 mm) and microvascular (100-200 um) coronary artery rings were isolated from the collateral-dependent LCX and non-occluded left anterior descending (LAD) arteries; conduit artery rings were also isolated from the right coronary artery (RCA). EDR to the endothelium-dependent agonists bradykinin (BK) and ADP was significantly enhanced in EX vs SED conduit arteries from both LCX ($p<.01$) and LAD ($p<.05$). Endothelium-independent relaxation to sodium nitroprusside was unaffected by EX. EDR to BK (30 nM) in LCX (but not LAD) microvessels was significantly enhanced and prolonged in EX vs SED groups ($p<0.001$). Inhibition of nitric oxide (NO) synthase (L-NAME; 100 uM) reversed EX-induced enhanced relaxation of LCX microvessels and partially blocked EX-induced increased EDR of LAD and LCX conduit arteries. In contrast to effects in LAD and LCX, preliminary studies suggest no effects of chronic LCX occlusion on EDR to BK (30 nM) of RCA, and the absence of EX-induced enhanced EDR in RCA. In summary, our combined data suggest that EX enhances endothelial NO-dependent relaxation of porcine coronary vasculature distal to chronic coronary artery occlusion and may thus improve blood flow regulation in the ischemic myocardial area-at-risk. Support: NIH-PO1-HL52490 & AHA

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HYPOXIA CAUSES INCREASED NITRIC OXIDE (NO) AND CYTOKINE PRODUCTION WITH A DELAYED T CELL RESPONSE

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Systemic hypoxia has multiple cellular effects. It has been suggested that NO blocks the response of T cells to stimulatory cytokines. We hypothesized that hypoxia activates the splenocytes and T cells to produce pro-inflammatory, as well as anti-inflammatory, cytokines and NO with a resultant delayed proliferative response. **METHODS** Male Sprague-Dawley rats (wt.=250-300grams) were placed in a chamber for 30 minutes at an F_iO_2 of 10%, with corresponding controls at room air. Rats were sacrificed immediately, or after one hour or 24 hours recovery at room air. Whole splenocytes and purified T cells were cultured with ConA, and supernatants were collected to measure NO, IL-2, IL-10, TNF-alpha, and IFN-gamma. Proliferation was assessed using MTT. **RESULTS** NO increased when rats were sacrificed immediately after hypoxia (6.425ng +/- 0.62, a 6-fold increase over controls, $p<0.002$), but decreased as recovery time increased. T cell proliferation was not stimulated in the hypoxic and 1 hour recovery groups, but there was a 65% increase in proliferation after 24 hours recovery, which correlated with the decrease in NO (Spearman correlation = -0.886, p=0.01). IL-10, IL-2, IFN-gamma, TNF-alpha increased immediately after hypoxia (4.9-fold, $p<0.05$; 2.3-fold, $p<0.02$; 4.5-fold, $p<0.02$; 1.8-fold, $p<0.05$, respectively). These cytokines decreased after 1 hour recovery,

however IL-10, IL-2, and IFN-gamma had a secondary increase in production at 24hours (1.5 fold, $p<0.02$; 1.3 fold, $p<0.02$; 1.3 fold, $p<0.002$ vs. 1hour recovery, respectively).
CONCLUSIONS These results suggest that hypoxia activates whole splenocytes/T cells and that, despite delayed T cell proliferation, T cell function and cytokine production remains intact. Our results also support the hypothesis that NO may be responsible for delayed T cell proliferation. The stimulus for the secondary rise in cytokine production is unclear.

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EPR analyzes of nitric oxide (NO) formation in the rats subjected to intestinal ischemia-reperfusion.

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NO formation during ischemia/reperfusion of intestine is associated with P-selectin- dependent adhesion of leukocytes in the liver microcirculation, in capillary leak and in the breakdown of intestinal mucosa. These conclusion were made on the base of the effects of NO sythase (NOS) inhibitors. Here we have used electron paramagnetic resonance (EPR) to investigate the time-course of nitric oxide generation in blood, intestine, liver, and lung and its susceptibility to inhibitors of nitric oxide synthase in intestinal ischemia-reperfusion. Ischemia was induced by clamping the superior mesenteric artery followed by release of the clamp after 1 h. NO formation was determined by measuring NO-heme complexes (NO-HEME), exhibiting a well defined EPR signal. Increased levels of NO-HEME in circulating blood were detected already at the end of ischemia. An additional increase of NO-HEME was observed during reperfusion. In intestine tissue the NO-HEME were also detected during ischemia and disappeared after starting the reperfusion, suggesting the release of NO into blood. Later, at 24 h of reperfusion, NO-HEME were detected in intestine tissue again. The fact that NO-HEME were not detected in other tissues (liver and lung) indicates that intestinal NO was not imported from non-ischemic tissues. Treatment with NG-monomethyl-L-arginine, a non selective inhibitor of nitric oxide synthase (given at 10 mg/kg i.v., 5 min prior to ischemia and 5 mg/kg i.v. 5 min prior to reperfusion), significantly reduced blood level of NO, but did not influence the concentration of NO in the tissue. In conclusion, the formation of NO during intestinal ischemia/reperfusion was detected directly both in blood and in intestine, indicating that intestine tissue is an important source of NO.

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EFFECT OF OXYGEN TENSION ON THE RELATIONSHIP BETWEEN iNOS ACTIVITY AND NITRIC OXIDE PRODUCTION

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INTRODUCTION: Macrophages (MΦ) exist in an environment of $\leq$ 40 torr O_2 *in vivo*. MΦ inducible nitric oxide synthase (iNOS) produces NO via oxidation of L-arginine. iNOS expression is reported to be upregulated during hypoxia, which should increase NO production. NO synthesis, however, also depends on adequate oxygen availability as a substrate. We investigated the effects of culture PO_2 on NO production and iNOS activity. **METHODS:** RAW 264.7 cells (a mouse MΦ cell line) were cultured on coverslips in a temperature controlled chamber with constant, controlled oxygen tensions (PO_2 1, 8, 24, 40, 80, 150, 356, and 677 torr). Cells were stimulated with 0.1 μg/ml LPS (E. coli 0111:B4) and 100 U/ml recombinant mouse interferon-γ. NO production was determined by measuring its stable metabolite nitrite by the Greiss reaction. Cell lysate iNOS activity was determined by the conversion of ^{14}C-Arginine to ^{14}C-Citrulline. Protein quantification was determined in cell lysates using the BioRad Protein Assay. Nitrite production and iNOS activity were normalized to protein concentration. **RESULTS:** NO production increased as a function of culture PO_2. Nitrite correlated with iNOS activity when PO_2 was greater than 40 torr (r^2=0.996, p= 0.0001). At oxygen tensions less than 40 torr, there was no correlation between nitrite and iNOS activity (r^2= 0.0234, p=0.8468). **CONCLUSIONS:** In contrast to the close relationship between NO production and iNOS activity observed in commonly used cell culture conditions, NO production is not directly related to iNOS activity in more physiologic environments. Extrapolation of results from non-physiologic cell culture environments to predict *in vivo* behavior may therefore be misleading.
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THE ROLE OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) IN HEART FUNCTION DURING ENDOTOXEMIA.

Paul H. Reinhardt, Wayne R. Giles, and *Paul Kubes. University of Calgary, Alberta, Canada, T2N-4N1. The purpose of this study was to determine the role of iNOS-derived nitric oxide (NO) in heart function during endotoxemia. Langendorff-perfused hearts from endotoxemic (lipopolysaccharide (10 mg/kg) for 6 hours) wild type (WT) C57BL-6 mice or inducible nitric oxide synthase-deficient (iNOS-KO) mice were monitored for left ventricular pressure (LVP) and maximal and minimal contractility (dP/dt_{max} and dP/dt_{min}). WT-endotoxemic but not WT-control or iNOS-KO hearts showed increases in mRNA for iNOS (RT-PCR) and nitrite production (Greiss reaction). In WT and iNOS-KO mice, endotoxemia resulted in decreases in LVP, dP/dt_{max}, and dP/dt_{min} relative to untreated controls. During isoproterenol challenge (0.1 μM), LVP, dP/dt_{max}, and dP/dt_{min} increased to the same levels in WT control and WT endotoxemic hearts. Endotoxemic iNOS-KO hearts did not respond as strongly to isoproterenol stimulation. As determined by histological analysis, neutrophil accumulation was 57% greater in the vasculature of endotoxemic iNOS-KO hearts than in WT endotoxemic hearts, while minimal numbers of neutrophils accumulated in control hearts. Endotoxemic hearts from iNOS-KO mice depleted of neutrophils (anti-neutrophil antibody-treated) responded to isoproterenol stimulation as well as control iNOS-KO hearts. Thus, NO from endogenous iNOS is cardiodepressive but does not affect the hearts ability to respond to β-adrenergic challenge. In the absence of iNOS, endotoxin-induced neutrophil accumulation becomes significant and affects the hearts ability to respond to β-adrenergic challenge.

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THE ROLE OF NITRIC OXIDE IN IGG IMMUNE COMPLEX-INDUCED LUNG INJURY IN RATS. T. P. Shanley[†], A. L. Salzman[†], C. Szabo[†], P. A. Ward[§]. [†]Division of Critical Care Medicine Children's Hospital Medical Center, Cincinnati, OH; [§]Department of Pathology, University of Michigan, Ann Arbor, MI.
Immune complex-induced acute lung injury in the rat is characterized by induction of iNOS by TNF-α and IL-1ß.

Tissue injury measured by lung leak was abrogated by non-selective inhibition of iNOS (L-NAME) and anti-cytokine therapy (α-TNF-α and α-IL-1ß Abs). It is hypothesized that the combination of NO with O2- to form the highly reactive oxidant, peroxynitrite leads to tissue injury. While NOS inhibition may be beneficial, non-selective inhibitors of iNOS have been associated with significant elevations in pulmonary artery pressures and increased mortality. Here, we further characterize the role of iNOS by determining the efficacy of selective iNOS inhibition on lung injury. Mercapto-ethylguanidine (MEG), a selective inhibitor of the inducible nitric oxide synthase and a peroxynitrite scavenger, was used. Conversely, the effect of inhaled NO on lung injury was also measured. Long-Evans rats (male, 250-300 gm) were treated with MEG (30 mg/kg, intraperitoneally based on previous studies), 30 minutes prior to initiation of IgG immune complex lung injury. Treatment with MEG resulted in a 46% ($p<0.01$) decrease in lung permeability, and a 52% ($p<0.05$) decrease in neutrophil numbers. NO_2/NO_3 levels were decreased by 25% suggesting that protection afforded by MEG may extend beyond iNOS inhibition. Although PMN infiltration was inhibited, BAL fluid MIP-2 (1.06 vs. 1.09 ng/ml) and MIP-1α (445 vs 502 pg/ml) levels did not differ. Rats underwent induction of IgG immune complex injury and were subsequently placed on mechanical ventilatory support with either 30% O_2 or 30% O_2 plus 40 ppm inhaled NO. Animals receiving NO had significantly more lung injury as reflected by increases in permeability index (1.68±.24 vs. 1.12±.28) and MPO (1.14 vs .88) Together, this data suggests that in a compartmentalized model of acute lung inflammation, selective inhibition of iNOS may be of benefit.

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ALTERATIONS IN TISSUE NITRIC OXIDE PRODUCTION AND INDUCIBLE NITRIC OXIDE SYNTHASE GENE EXPRESSION DURING POLYMICROBIAL SEPSIS. P. Shieh*, M. Zhou*, I.H. Chaudry, and P. Wang. Brown University School of Medicine and Rhode Island Hospital, Providence, RI 02903.

Polymicrobial sepsis is characterized by an early, hyperdynamic phase (i.e., 2-10 h after cecal ligation and puncture, CLP) followed by a late, hypodynamic phase (20 h after CLP). Although studies indicated that plasma levels of nitrate/nitrite [NO_3^-/NO_2^-; stable products of nitric oxide (NO)] increase at 10-20 h after the onset of sepsis, it remains unknown which organs are responsible for producing the increased NO under such conditions. To determine this, male adult Sprague-Dawley rats were subjected to sepsis by CLP followed by fluid resuscitation. At 10 h after CLP (a late stage of the hyperdynamic phase of sepsis), the liver, small intestine, kidneys, heart, lungs and thoracic aorta were harvested. Tissue levels of NO_3^-/NO_2^- were determined using colorimetric assays. In addition, inducible NO synthase (iNOS) gene expression was examined at 10 h after CLP by RT-PCR technique. The tissue levels of NO_3^-/NO_2^- (nmol/g; n=7-9/group; mean ± SEM) were as follows:

	Liver	S. Intestine	Kidneys
Sham	12.17± 2.78	5.51±1.73	10.15±3.01
CLP	23.64±1.70*	25.92±3.12*	25.61±2.38*
	Heart	**Lungs**	**Aorta**
Sham	24.74±3.02	22.22±3.24	104.37±42.67
CLP	37.07±3.66*	28.66±3.82	139.68±18.35

(Unpaired Student's t-test: *$p < 0.05$ versus respective sham)

These results indicate that NO_3^-/NO_2^- increased significantly in the liver, small intestine, kidneys and heart at 10 h after the onset of sepsis. Moreover, iNOS mRNA was upregulated in the above tissues. Thus, the liver, intestine, kidneys and heart appear to be important sites responsible for producing the increased NO during polymicrobial sepsis. Since we have previously demonstrated that constitutive NOS is downregulated during sepsis, the overproduction of NO is most likely caused by upregulation of iNOS gene expression under such conditions (Supported by NIH GM 53008).

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EFFECTS OF HYPERBARIC OXYGENATION (HBO) ON NO/TNF-α FORMATION AND SURVIVAL IN EXPERIMENTAL ENDOTOXIC SHOCK

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Introduction: HBO has been used in a variety of clinical conditions, without scientific evaluation of the underlying mechanisms. The excessive formation of inflammatory mediators, including nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α) that occurs secondary to the stimulation in a variety of cells has been thought to contribute to the development of multiple organ failure during endotoxic or septic shock. We investigated the effects of HBO on endotoxin-induced formation of NO and TNF-α in rats. **Material and Methods:** One h after endotoxin challenge (5 $mg.kg^{-1}$ body weight i.v.) animals were exposed to 100% O2 at 3 atm for two hours. Immediately thereafter the experiment was terminated (3 h after LPS challenge). **Results:** We found a significant increase in Ca2+-dependent and Ca2+-independent NO synthase (cNOS and iNOS) activity in lung tissue homogenates at 3 h after endotoxin challenge. HBO for two hours did not influence the cNOS and iNOS activity as compared to the controls (0.27±0.27 and 7.56±2.91 vs. 0.49±0.33 and 8.15±2.06 $pmol.min^{-1}.mg^{-1}$protein). Similarly, plasma levels of nitrite/nitrate did not differ between HBO treated animals and controls (69.35±13.37 vs. 82.29±17.34 $nmol.ml^{-1}$). At 3 h after endotoxin injection, also the endotoxin-induced increase in plasma TNF-α · concentrations in the controls was not significantly affected by HBO treatment (4.9±1.9 vs. 3.1±1.7 $ng.ml^{-1}$). However, HBO treatment, even one hour after endotoxin challenge protected rats during the experiment. While 4 out of 9 controls died between 1 and 3 h, all HBO treated animals (n=5) survived. **Conclusion:** Our data indicate a protective effect of HBO against endotoxic shock, at least at the early stage (during HBO), which appears to be due mechanisms other than the inhibition of NOS activity in the lung tissues or a decrease in plasma levels of NO and TNF-α.

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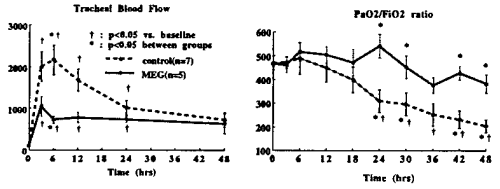
EFFECT OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) INHIBITION ON SMOKE INHALATION INJURY IN SHEEP **K.Soejima*, L.D. Traber, and D.L.Traber , Univ. Texas and Shriners Burns Institute, Galveston, TX 77555-0833**

We hypothesised that overproduction of NO from iNOS in the airway contributes to significant increases in airway blood flow after smoke inhalation and the iNOS-derived NO plays a major role on the loss of hypoxic pulmonary vasoconstriction. We tested this hypothesis by administering mercaptoethyl-guanidine (MEG) which is a selective iNOS inhibitor and peroxynitrite scavenger to sheep insufflated with smoke.

Sheep (n=14) were surgically prepared for chronic study. After 5-7 days recovery, the sheep received 48 breaths of cotton smoke. The animals were randomised into 2 groups: MEG group (30mg/kg MEG was given 1 h after smoke and then every 8 h for 41 h, n=7) and control group (0.9% NaCl, n=7). All animals were mechanically ventilated (tidal volume 15ml/kg, PEEP 5 cm H_2O and FiO_2 was adjusted to maintain SaO_2>90%). Airway blood flow was measured using colored microsphere.

With smoke airway blood flow rose and PaO_2/FiO_2 fell. In the MEG group, rise in airway blood flow and the fall in PaO_2/FiO_2 ratio were attenuated. The average arterial plasma

conjugated dines were significantly lower in the MEG group (0.16±0.01 MEG group versus 0.28±0.05 control group).



We conclude that iNOS inhibition has a beneficial effect in inhalation injury.

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LPS-TOLERANCE MODULATES THE EFFECTS OF ACUTE ALCOHOL ON HEPATIC NO PRODUCTION. J.A. Spitzer and J.J. Spitzer, Dept. of Physiol., LSU Med. Ctr. New Orleans, LA 70112.

Previous work indicated that acute alcohol intoxication may alter the redox state of the liver involving both superoxide and nitric oxide (NO) production. We tested the hypothesis that the LPS-tolerant state alters the synthesis of NO by hepatocytes (HC) and Kupffer (KC) cells following acute alcohol intoxication and thus contributes to changes in the redox state. The time course of these changes and the existence of gender differences were also investigated. Age matched ♂ and ♀ rats were tolerized by 0.45 mg/kg i.v. LPS 2, 3 or 5 days prior to an *in vivo* EtOH infusion for 3h (LPS-EtOH group). Control animals were saline-pretreated and EtOH infused (sal-EtOH). At the end of the EtOH infusion HC and KC were cultured for 20h in the absence and presence of LPS or IFN for subsequent measurement of nitrite release (as an index of NO production). In the 2, 3 and 5 day tolerant animals basal NO release by HC was less in the LPS-EtOH, than in the sal-EtOH group both in ♂ and ♀ rats. HC of ♀ (but not of ♂) rats were primed for *in vitro* stimulation in the LPS-EtOH group at 2 days. At 3 and 5 days priming disappeared in females and became evident in males. KC taken from ♂ rats showed increased NO basal release in the LPS-EtOH group at 2, 3, and 5 days and these cells were also markedly primed. Similar changes in KC of ♀ rats were observed only at 3 and 5 days of tolerance. It appears that tolerance is associated with an increased ability of both parenchymal and non-parenchymal cells to produce NO after EtOH. The time course of these changes however, is different in the two cell types and is also subject to gender differences. The enhanced hepatic NO production in LPS-EtOH treated rats is likely to contribute to suppressed superoxide release under such conditions (A.P. Bautista & J.J. Spitzer. 1996),♂ and it may serve as a protective mechanism against potential oxidative injury. (Supp. by AA 09803 and ONR grant N00014-98-1-0460)

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PEROXYNITRITE (PN) INDUCES ENTEROCYTE APOPTOSIS IN VITRO: IMPLICATION FOR NO-MEDIATED GUT BARRIER FAILURE. J. Upperman *, C. Friend *, E. Nadler*, R. Hoffman*, H.R. Ford. Children's Hospital of Pittsburgh, Pittsburgh PA, 15213

Purpose: We have previously shown that gut barrier failure following necrotizing enterocolitis or endotoxemia is associated with upregulation of inducible nitric oxide synthase, 3-nitrotyrosine residues (the PN footprints) and apoptosis in the ileum. The mechanism of PN-mediated gut barrier failure remains elusive. This *in vitro* study examines PN-mediated epithelial cell injury. **Methods**: IEC-6 cells were incubated with 12.5 μM PN or PBS for 60 minutes then recovery of 6,12, 18 or 24h in culture media. Apoptosis was measured by FACS using the propidium iodide method and electron microscopy. We also tested the ability of 3-aminobenzamide (3AB) (1mM), an inhibitor of poly-ADP ribose synthetase, pretreatment to alter the response of IEC-6 cells to PN. **Results**: PN induced apoptosis in IEC-6 cells in a time-dependent fashion(Fig. 1).A 60 minute exposure to PN followed by 18h recovery period resulted in significant apoptosis (24±4 %) in IEC-6 cells compared to PBS (2.7±.5%); $p<0.015$. There was no significant-difference between PN and PN + 3AB (24±4 v 17±4 %). Higher doses resulted in necrosis. Electron microscopy confirmed the injury. **Conclusions**: PN directly injures small intestinal epithelial cells. Thus, inhibition or scavengers of nitric oxide or PN may ameliorate gut barrier failure after endotoxemia.

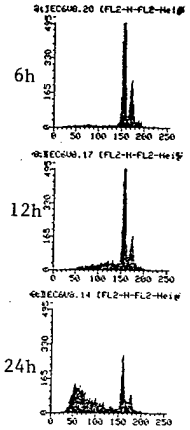

Fig. 1
Kinetics of PN-induced Apoptosis

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DECREASED NITRIC OXIDE PRODUCTION BUT INCREASED TISSUE WATER CONTENT IN HEART, LIVER AND KIDNEY OF RATS WITH DELAYED FLUID RESUSCITATION OF SEVERE BURN. B. Yu, H. Shen, Y. Chen, J. Horton, Z. Xia Changhai Hospital, Shanghai 200433, CHINA; UTSWMC, Dallas, TX 75235

Nitric oxide (NO) prevents neutrophil adhesion to endothelial cells; altered or insufficient NO production after burn trauma may contribute to endothelial dysfunction. This study was designed to identify the effects of delayed fluid resuscitation of severe burn trauma on NO production as well as fluid flux across membranes (indicated by tissue water content, TWC). **METHODS:** Sprague-Dawley rats were randomly divided into burn with early fluid resuscitation (BE) and burn with delayed fluid resuscitation (BD) groups. A 3°scald burn over 30% TBSA was produced; fluid resuscitation (Parkland formula) was initiated 30 min (BE group) or 7 hrs (BD group) postburn. Rats were sacrifice 8 hrs postburn and tissue collected. Tissue NO levels were expressed as nmol/mg protein, and TWC determined from wet/dry wts. **RESULTS**: NO in heart, liver and kidney decreased significantly with BD resuscitation compared to rats given BE resuscitation; TWC increased in BD compared to BE group (Table 1). **CONCLUSION**: Delayed fluid resuscitation of severe burn injury may alter endothelial function due to impaired NO synthesis; endothelial dysfunction and altered microvascular permeability may contribute to postburn organ injury.

	Heart	Liver	Kidney
NO			
BE group	2635±255	2947±219	2198±219
BD group	1624±70**	1461±67**	1665±86**
TWC			
BE group	79±2	70±2	77±2
BD group	82±2*	74±3*	81±2*

*$p<0.05$, ** $p<0.01$ when comparing to BE group

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THE ROLE OF ARTERIOLAR MEMBRANE POTENTIAL AND NO IN VASCULAR HYPOREACTIVITY DURING SEVERE SHOCK

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The goal of present study is to elucidate the mechanism of vascular hyporeactivity in severe shock. Irreversible hemorrhagic shock of rat was reproduced and the response of spinotrapezius muscle to norepinephrine (NE) was tested. The resting membrane potential of isolated arterial strips was measured with a microelectrode. The change of membrane potential in the isolated arteriolar smooth muscle cell post addition of NO was determined with fluorescent probes under confocal microscope. The K_{ATP} channel of smooth muscle was detected with patch clamp method. It was shown that the resting potential was increased from –36.7±6.3mV of control value to –51.0±9.1mV with the NE threshold increased to 15 times more than prehemorrhage value 2h post-shock. The membrane hyperpolarization of smooth muscle existed only in the late stage of shock, which was closely related to vascular hyporeactivity (correlation coefficient 0.96, $p<0.01$). The addition of NO led to exacerbate the membrane hyperpolarization with negative resting potential increased by 70.1±7.6%. Single K_{ATP} channel conductance, mean open time and open probability was increased and treatment with glybenclamide partially restored the membrane hyperpolarization and vasoreactivity in late stage of shock. The study indicates that hyperpolarization of smooth muscle enhanced by NO release contributes to vessel hyporeactivity and the open of K_{ATP} channel is one of the reasons for the hyperpolarization in severe shock.

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IL-10 AS A MEDIATOR OF ACTIVATION INDUCED LYMPHOCYTE APOPTOSIS IN SEPSIS.

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Recent studies suggest that increased activation induced lymphocyte apoptosis (*act*-A_o) is detected in septic mouse spleno-cytes (SPL) which may contribute to lymphocyte immune dysfunction (↓IL-2, ↓ IFN-γ). Furthermore, these changes in SPL response are associated with an increased release of the immuno-suppressive cytokine, IL-10. However, it is unknown if this increase in *act*-A_o is regulated by IL-10. To assess this, SPLs were harvested from C3H/HeN mice at 24 h after the onset of polymicrobial sepsis [cecal ligation and puncture (CLP)] or Sham-CLP (Sham) and then stimulated 24 h with 2.5 μg Con A/ml in the presence or absence of antibody to IL-10. When SPLs were assessed for *act*-A_o by flow cytometric cell cycle analysis, it was seen that antibody to IL-10 blunted the CLP induced increase in % *act*-A_o (Sham, 20.4±3.1; CLP, 27.9±2.1*; CLP/anti-IL-10 21.2±2.1; ±SEM, 5/grp, Mann-Whittney U, *$p<0.05$ vs Sham). To further determine that the effect of IL-10 is not only on SPL *act*-A_o but also on IL-2/IFN-γ release, we assessed the ability of SPLs from Sham or CLP IL-10 knockout mice (C57-BL6/J-*IL10 -/-*) or their respective controls (C57BL6/J) to undergo *act*-A_o and elaborate cytokines (ELISA), in additional studies.

Strains	Group	% *act*-A_o	IL-2 (ng/ml)	IFN-γ (ng/ml)
C57BL6/J	**Sham:**	33.5±4.3	1.5±0.1	1.5±0.3
	CLP:	48.9±7.2*↑	0.9±0.2*↓	0.6±0.2*↓
C57BL6/J-	**Sham:**	23.0±6.2	1.7±0.2	1.8±0.1
IL10 -/-	**CLP:**	31.2±5.5	1.9±0.3	1.7±0.3

(*$p<0.05$ vs Sham, Mann-Whittney U, ±SEM, n=5/grp)

The results indicate that while CLP in the control mice increased *act*-A_o while decreasing IL-2 release, deficiency of IL-10 depressed *act*-A_o and improved SPL IL-2 release. Taken together, these data indicate that the Th2 cytokine IL-10 not only serves to actively suppress septic lymphocyte Th1 responses (IL-2/IFN-γ release), thus compromising immunity, but eventually also drives these cells into apoptotic cell death.

(Supported by NIH GM53209)

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THE INTERACTION OF HSP70 WITH RIBOSOMES IS INVOLVED IN THE PROTECTION OF PROTEIN SYNTHEIS DURING HEAT SHOCK IN STRESS TOLERANT CELLS.

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Expression of heat shock or stress proteins (hsps) is an universal response to stress. The presence of these proteins has been correlated with the protection of cells from different kinds of stresses, "stress tolerance." We have postulated that stress tolerance is achieved by the stabilization of different cellular structures and pathways. Indeed, proteins synthesis is preserved during heat shock in stress tolerant cells. Previous studies have shown the association of Hsp70 with translating ribosomes. This interaction has been further characterized. Hsp70 binds to free 40S ribosomal subunits. This binding was observed in cells incubated at 37°C or 43°C. The interaction between Hsp70 and ribosomes is salt resistant, suggesting that Hsp70 is not interacting with translation factors which are weakly and transiently associated to ribosomes. This observation suggests that binding of Hsp70 to 40S ribosomal subunits has a high affinity. Ribosomal subunits containing Hsp70 seem to be preferentially used for the translation of messages during stress. Protein synthesis is preserved during heat shock after transfection with the Hsp70 gene, suggesting that this protein is required for the protective effect. Moreover, partial protection of protein synthesis is observed simultaneously with the new synthesis of Hsp70. These results suggest that the binding between Hsp 70 and 40S ribosomal subunits is necessary for the protection of protein synthesis observed in thermotolerant cells. Moreover, we speculate that the interaction of Hsp70 with ribosomes is part of a mechanism to guarantee the rapid and abundant synthesis of hsps during stress which is require for the subsequent protection of the cell.

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p53-INDUCED APOPTOSIS IS MEDIATED BY AN INCREASE IN THE PRODUCTION OF REACTIVE OXYGEN SPECIES (ROS) FOLLOWED BY INTRACELLULAR CALCIUM MOBILIZATION.

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p53 has been shown to play an important role in cell death associated with ischemia reperfusion injury. ROS have been proposed to act as intermediates for p53-induced apoptosis and an active role for calcium in the initiation of apoptosis has been suggested as well. To investigate the roles of ROS and calcium in p53-dependent apoptosis, we measured ROS production, intracellular calcium [Ca2+]i and apoptosis in Li-Fraumeni 041 p53 Tet-Off cells expressing p53 under the control of a tetracycline-regulated promoter. Apoptosis was determined using Hoechst stain. ROS was measured as dichlorofluorescein (DCF) fluorescence and [Ca2+]i

was determined by Fluo-3 AM using confocal microscopy. Expression of p53 resulted in increases in DCF fluorescence and [Ca2+]i followed by induction of apoptosis. The ROS increase was maximal 48 hrs after p53 induction, while [Ca2+]i peaked at 72 hrs. The rate of apoptosis was maximal 4 days after induction of p53. Increased ROS production and apoptosis were inhibited by N-acetyl cysteine and rotenone, a specific mitochondrial complex I inhibitor. These data show that p53-induced apoptosis is mediated by an increase in ROS production followed by a rise in [Ca2+]i. It is hypothesized that mitochondria are likely to play a key role in p53-induced ROS production which is then followed by calcium mobilization leading to apoptosis. (Supported by NIH DK15440, and an intramural grant from the University of Maryland School of Medicine.)

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MILD AND FULMINANT SEPSIS INDUCE DIFFERENT PATTERNS OF BILE ACID TRANSPORTER TRANSCRIPTION AND HISTOLOGIC CHANGES IN RATS. Patrick K. Kim*, Jodi Chen*, Kenneth M. Andrejko*, and Clifford S. Deutschman, University of Pennsylvania, Philadelphia, PA 19104.

Jaundice and cholestasis are the hallmarks of hepatic dysfunction in sepsis. Transport of bile salts and bilirubin across hepatocytes and into bile is a function of the bile acid transporters ntcp and mrp2. We have shown that sepsis in rats and mice decreases transcription of several liver-specific genes. We hypothesize that (1) cecal ligation and single puncture (CLP) and cecal ligation and double puncture (2CLP) alter transcription of ntcp and mrp2, and (2) persistent loss of ntcp and mrp2 transcription in 2CLP results in morphologic changes consistent with cholestasis. NIH guidelines were followed. Male Sprague-Dawley rats underwent CLP, 2CLP, or sham operation (SO). Hepatic nuclei were harvested at 0, 3, 6, 16, 24, 48, or 72 hrs, and nuclear run-on was performed. Tissue was fixed and stained with hematoxylin and eosin. There was no mortality after SO or CLP. Survival after 2CLP was 50% at 24 hrs, 25% at 48 hrs and occasional (<5%) at 72 hrs. Nuclear run-on showed significantly decreased transcription of ntcp and mrp2 three hrs after CLP or 2CLP. 72 hrs after CLP, transcription of both ntcp and mrp2 had increased to 41% of baseline, but at 24 hrs or later after 2CLP, transcription of ntcp and mrp2 remained negligible. CLP caused only mild changes in hepatic architecture (sinusoidal narrowing, disruption of arrays), while 2CLP resulted in increased bile duct narrowing and obstruction, as well as intracellular fat and bilirubin deposition. We conclude that (1) transcription of ntcp and mrp2 is reversibly depressed in mild sepsis but irreversibly depressed in fulminant sepsis, and (2) persistent loss of bile acid transporter transcription in fulminant sepsis results in cholestasis, a morphologic indication of hepatic dysfunction.

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PHOSPHORYLATION OF HSP27 LEADS TO ALTERATIONS IN CYCLIC NUCLEOTIDE DEPENDENT RELAXATION. L. Knoepp*, J.S. Mondy, C. Brophy*, Medical College of Georgia, Augusta, GA 30912.

It has been suggested that the small heat shock protein HSP27 modulates contractile responses of vascular smooth muscle (VSM). Cellular stress in the form of arsenite leads to increases in the phosphorylation of HSP27. We hypothesized that stress would lead to alterations in the contraction and/or relaxation of VSM. **Methods:** Bovine VSM strips were equilibrated in buffer and physiologic responses were determined in a muscle bath. The strips were treated with buffer alone (control, C), arsenite (0.5mM, 30", AS), in the presence or absence of SB203580 which inhibits HSP27 phosphorylation. The VSM strips were equilibrated for two hours and then treated with the contractile agonist serotonin (5HT, 1μM, 10") followed by the adenylate cyclase activator sodium nitroprusside (SNP, 10^{-5}M). The contractile response to 5HT is expressed as the % of contraction to an initial high KCl contraction (110 mM) and the relaxation response to SNP as % change from 5HT, +/- SEM, * = $p < 0.05$, ANOVA, n = 8. **Results:** Stress alone (AS) did not alter contractile responses (Figure 1) but decreased the relaxation response (Figure 2). The effects of arsenite were inhibited by SB203580. **Conclusions:** Stress does not effect contraction responses but impairs cyclic nucleotide-dependent relaxation. Inhibition by SB203580 suggests that the effects of stress are mediated by HSP27 phosphorylation.

Figure 1

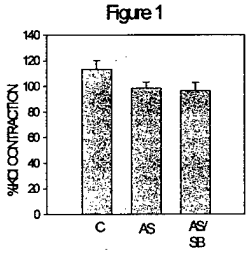
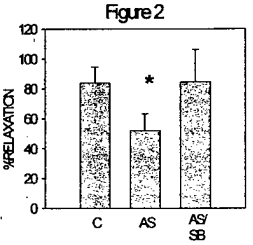


Figure 2



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α-GLUTATHIONE S-TRANSFERASE: A SENSITIVE INDICATOR OF HEPATOCELLULAR DAMAGE DURING POLYMICROBIAL SEPSIS. D.J. Koo*, M. Zhou*, I.H. Chaudry, and P. Wang. Brown University School of Medicine and Rhode Island Hospital, Providence, RI 02903.

Although studies have examined the liver enzyme α-glutathione S-transferase (αGST) as a marker of hepatic injury following hemorrhagic shock, it remains unknown if this enzyme is also useful for determining hepatocellular damage during the early stage of sepsis. To study this, male rats (~300g) were subjected to polymicrobial sepsis by cecal ligation and puncture (CLP) or sham operation followed by fluid resuscitation. Systemic blood samples were taken at 2, 5, 10, or 20 h after CLP or sham operation. Plasma levels of αGST (μg/L) and lactate (mg/dL) were determined using an enzyme immunoassay and enzymatic assay, respectively. Additional animals were utilized to examine morphological alterations in hepatocellular ultrastructure during sepsis using electron microscopy. The data (mean ± SE, n=6-8/group) are as follows:

		2 h	5 h	10 h	20 h
αGST	Sham	29 ± 6	32 ± 8	29 ± 6	32 ± 10
	CLP	28 ± 3	112 ± 19*	159 ± 33*	229 ± 26*
Lactate	Sham	26 ± 6	17 ± 2	23 ± 3	22 ± 2
	CLP	32 ± 6	22 ± 3	28 ± 2	47 ± 5*

(Student's t-test; * $p < 0.05$ vs. Sham at each time point)

The results indicate that although αGST did not change at 2 h after CLP, it was elevated by 249% at 5 h after the onset of sepsis and continued to increase throughout the septic episode. In contrast, plasma lactate did not significantly increase until 20 h after CLP. Moreover, electron microscopy revealed significant changes in hepatocellular morphology at 5 and 20 h after CLP which were indicative of hepatocellular injury. Since the elevation in plasma αGST and alterations in hepatocyte ultrastructure occur at 5 h after CLP, this cytosolic liver enzyme appears to be capable of detecting early hepatocellular damage during sepsis. The fact that plasma αGST levels increased earlier than plasma lactate and liver transaminases (increased at 10 h after CLP or later) suggests that αGST is a more sensitive indicator of liver injury and should be utilized in monitoring hepatocellular damage during the progression of polymicrobial sepsis (Supported by NIH GM 53008).

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ALTERATIONS IN THE ACTIVITIES OF T CELL NUCLEAR FACTORS, NFAT AND AP-1, AFTER BURN INJURY

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Studies from several laboratories have shown altered IL-2 production and proliferation by T cells harvested from animals with burn injury. In this study, we have evaluated whether IL-2 production and proliferation response alterations are accompanied by altered activity of the T cell nuclear factor specific to IL-2 transcription, NFAT, and/or the ubiquitous T cell nuclear factor AP-1. Splenic T cells were isolated from control or burn(3rd degree, 25% TBSA) rats. T cells were stimulated with concanavalin A(ConA), and the DNA binding activity of transcription factors NFAT and AP-1 were measured using electorphoretic mobility shift assays. The results were:

	Control	Burn
NFAT	169.1± 16.5*	133.2± 17.0
AP-1	158.0±19.6	115.5±20.4

*Mean±SE values in arbitrary densitometric units.

The data show that the ability of NFAT and AP-1 ologonucleotides to bind to nuclear extracts from T cells of burn injured rats decreased significantly. The lower activities of the T cell nuclear factors correlate with the decrease in IL-2 expression in T cells of burn injured rats. (supported by NIH grants GM 53235 and GM 568501).

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NUCLEAR FACTOR KAPPA B (NF-κB) DOES NOT MODULATE SEPTIC RESPONSES IN MOUSE LIVER

Tania Potts*, Nichelle R. Raj*, Patrick Kim*, Clifford S. Deutschman. Department of Anesthesia, University of Pennsylvania

NF-κB, a ubiquitous inducible transcription factor that modulates immune and inflammatory responses, is activated by tumor necrosis factor alpha (TNFα) and interleukin-1β (IL-1β). These cytokines have been implicated in septic responses. Cytoplasmic NFκB is maintained in an inactivated state by binding of an inhibitor, IκBα. TNFα and IL-1β initiate phosphorylation, ubiquitination and proteolysis of IκB, allowing free NF-κB to translocate to the nucleus, bind to DNA and stimulate transcription. Free IκB can later migrate into the nucleus, bind to nuclear NF-κB and rescue it into the cytoplasm. We hypothesize that sepsis secondary to cecal ligation and double puncture (2CLP) activates the NF-κB/IκBα pathway. Protocols adhere to NIH guidelines. Male C57Bl/6 mice underwent 2CLP or sham operation (SO). Mice were sacrificed at 0, 3, 6, 16, 24, and 48 hours, hepatic tissue was processed for nuclear and cytoplasmic protein and Western Blot analysis was performed. These studies revealed an initial dissociation of cytoplasmic IκB can from NF-κB, translocation of NF-κB to the nucleus, secondary movement of IκB into the nucleus and retrieval of NF-κB to the cytoplasm. Responses, however, were the same in both 2CLP and SO. Therefore, we conclude that NF-κB does not modulate septic-specific responses in mouse liver.

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COMPARATIVE ROLE OF ANTIOXIDANTS FOR MODULATION OF AP-1 AND NF-κB AND THEIR ROLE FOR HEME OXYGENASE-1 GENE EXPRESSION IN THE LIVER AFTER HEMORRHAGIC SHOCK

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Hemorrhage and resuscitation (H/R) lead to the activation of transcription factors and subsequent induction of stress genes. We have previously shown, that induction of the stress protein heme oxygenase(HO)-1 can be inhibited by antioxidants (1), contributes to maintenance of hepatic blood flow (2) and subserves a protective role after H/R (3). Aim of the present study was to assess the role of reactive oxygen species (ROS) for activation of the redox sensitive transcription factors NF-κB and AP-1 and their contribution to HO-1 gene expression after H/R in the liver. Sprague-Dawley rats were subjected to H/R (MAP = 40mmHg for 1h, followed by 5 h resuscitation). In some experiments the antioxidant Trolox (6mg/kg iv) was administered at the onset of resuscitation. Expression of HO-1 was studied by standard Northern analysis. Nuclear protein extracts were isolated for electrophoretic mobility shift assay of NFκB and AP-1. Sham and H/R led to a similiar and substantial activation of NF-κB compared to unmanipulated controls which was not modulated by Trolox. Activation of AP-1 was only observed after H/R and attenuated by Trolox (sham: 8±1.2; H/R: 100±17.6; Trolox: 44±12.5; densitometric units; $p<0.05$). A substantial increase in HO-1 steady state transcript levels was observed over sham operated controls which was diminuished by administration of Trolox (sham: 1.6 ± 0.24; H/R: 6.7 ± 0.31; Trolox:: 3.4 ± 0.24 densitometric units; $p<0.05$). While NF-κB is activated easily even by sham operation, activation of AP-1 occurs only after H/R and is in part dependent on ROS. Activation of AP-1 correlates with induction of HO-1, an antioxidative stress protein with known binding sites for AP-1 in the promoter/enhancer region. (supported by DFG grant (Ba1601/1-2). (1)Rensing H et al.: Shock 9: S11 (2)Bauer M et al.: Am.J. Physiol. 271:G929-935, 1996. (3)Rensing H et al.: Crit Care Med in press 1999.

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IDENTIFICATION OF A NO REGULATED TRANSCRIPTION FACTOR USING A NOVEL MOTIF DIRECTED DIFFERENTIAL DISPLAY METHOD.

U. Schaefer, A. Schneider and E. Neugebauer. Biochem & Expt. Division, II. Dep. of Surgery, University of Cologne

NO has been established as an important second messenger mediating cellular and physiological responses during inflammation. However, the elucidation of NO mediated gene regulation during inflammatory processes has only reached an initial stage. In order to identify transcription factors which are regulated during early inflammatory processes we have developed a novel differential display (DD) method using motif primers and nested amplifications. Method: Human umbilical vein endothelial cells (HUVECs) were grown to confluence and exposed to histamine, histamine + NOS inhibitor and a nitric oxide donor. Total RNA was isolated at different time points. The DD method published by Liang and Pardee was modified. A polydT_{12} downstream primer was constructed including a 3' dN_{10} sequence allowing nested PCRs. The upstream primer was directed to Kruppel-like zinc finger motifs. Differentially expressed cDNAs were cloned,

sequenced and analysed by Northern Blot Hybridisation. Result: The motif directed DD lead to the identification of a differentially expressed 720 bp cDNA band. Cloning and sequencing analyses revealed the cDNA fragment to encode the 3' region of the human zinc finger protein (HZF2). Northern blot analysis using the cDNA fragment as a hybridization probe revealed a hybridization signal of about 2.5 kb. This size was consistent with the HZF2 mRNA size. Within 2 hours of histamine stimulation the HZF2 mRNA level showed a two-fold increase as compared to unstimulated HUVECs. Inhibition of NOS leads to attenuation of the histamine induced increase in HZF2 mRNA levels. An increase of HZF2 message is also observed during incubation of HUVECs with a NO donor. Conclusion: These results demonstrate the motif directed DD to be a promising method for the identification of gene groups with motif related functions. The zinc finger protein HZF2 was one of 42 genes isolated from the human monoblast cell line U-937. We were able to show a possible role of one of the Kruppel-related zinc finger proteins in early inflammation.

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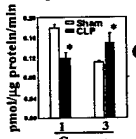
THE ROLE OF CASPASES IN MACROPHAGE CELL DYSFUNCTION AND APOPTOSIS DURING SEPSIS. C.S. Chung*, G.Y. Song*, L.L. Moldawer#, I.H. Chaudry, and A. Ayala. Dept. Surgery, Brown Univ. Med. Sch/R.I. Hospital, Providence, RI 02903. # Dept. Surgery, Univ. Florida, College Med., Gainesville, FL, 32610.

Recent studies indicate that macrophages (Mϕ) isolated from septic mice exhibit a marked loss of important immune functional capacity (i.e., decreased inducible IL-1 and IL-6 release) which is associated with significant increase in Mϕ apoptosis (A_o), a form of cell suicide. However, the mechanism responsible for these changes as well as the interactions between cytokine productive and apoptotic processes in Mϕ is poorly understood. In this respect, while caspases are key components of the cell death machinery, their role in Mϕ induced A_o seen during sepsis is not established. The aim of this study, therefore, was to determine if the increase in cell death seen in peritoneal Mϕ (pMϕ) of septic mice is due to altered caspase activity. To study this, male C3H/HeN mice were subjected to cecal ligation and puncture (CLP) or sham operation. 24 h later, pMϕ cultures were established, stimulated with lipopolysaccharide (LPS) and harvested for A_o determination by cell death ELISA or PI-cell cycle analysis. Concomitantly, IL-1β release (by ELISA), caspase enzyme activity (using caspase 1 or 3 specific substrates) and caspase family gene expression (by RNase protection assay) were also assessed.



Group	Indices of A_o: Cell death ELISA(OD)	PI Stain (% A_o+)	IL-1β (ng/ml)
Sham	0.45±0.05	6.9±1.0	0.42±0.13
CLP	0.58±0.02*	33.3±6.6*	0.001±0.0005*

* P<0.05 vs. sham; Mann-Whitney U-test, mean±SE, n=4-7/group

The results indicate that the activation of caspase 3 family and upregulated caspase 3 gene expression correlated with the increase in A_o after LPS stimulation in pMϕs from septic mice. However, caspase 1 activity was downregulated, which is consistent with decreased IL-1β release seen after CLP. This implies that caspase 3 family members, but not caspase 1, play a central role in increasing inducible Mϕ A_o seen during sepsis. Thus, the use of specific caspase antagonists may have potential salutary effect on the immune responses in phagocytes during sepsis. (NIH GM 53209)

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DEVELOPMENTAL CHANGES IN PERITONEAL MACROPHAGE CYTOKINE RESPONSE Kevin P. Lally, Hasen Xue, Julie Thompson Univ. of TX Houston, Dept. of Surgery, 6431 Fannin, #5.258, Houston, TX 77030.

Neonates have a marked age-related susceptibility to death from sepsis. While the neonate is felt to be immunocompromised, early septic death is due to excess cytokine release. We hypothesized that the neonatal macrophage would respond differently than adults. Peritoneal macrophages were harvested from litters of C3H/HEN mice at different ages using cold RPMI lavage, with adult controls. The cells were plated at 10^5 cells per well, allowed to adhere for 1 hour and gently washed. Cells were stimulated with 10ug/ml of LPS for 24 hours, negative controls received media alone. Supernatant cytokine levels were measured using ELISA kits for murine TNF*a*, IL-10 and KC. At least 5 sets of litters were used for each age tested. Data were analyzed using an unpaired t test. In separate experiments quantitative PCR for murine TNF mRNA was performed on 1-day-old and adult mice. There were significant age-related differences in TNF*a* release with a marked response at 1 day of age and a nadir at 14 days. The stimulated IL-10 response in the 14-day-old group was significantly higher than at other ages and there was no difference in KC response by age. The 1-day-old mice had an 8-fold greater mRNA response by PCR. There was a marked increase in baseline activity for all cytokines in the newborn mice. We conclude that there are significant developmental differences in the neonatal macrophage response. The increase in baseline activity in newborns suggests some level of peripartum stimulation as well. Appropriate strategies to treat neonatal sepsis may require tailoring to specific gestational ages.

		1 day	7 days	14 days	21 days	Adult
TNF*a*	Control	122	60	34	9	45
(pg/ml)	LPS	2638#	642	289*	338*	647
IL-10	Control	13	2	0.7	5	0.4
(pg/ml)	LPS	67	63	204**	92	76

#p<0.001 vs. all others, *p<0.001 vs. adult controls, **p<0.001 vs. others.

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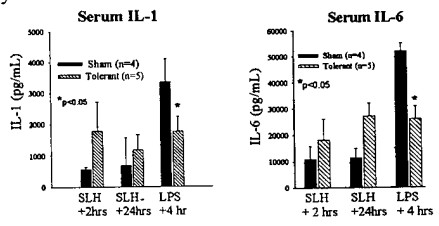
INDUCTION OF TOLERANCE BY SUBLETHAL HEMORRHAGE ALTERS LPS STIMULATED IL-1 AND IL-6 PRODUCTION C Mendez*, A Kramer*, V Wong*, J Norman*, L Carey*, (Spon: R Maier) USF, Tampa, FL. 33612

It has been postulated that the immune response to hemorrhagic shock worsens outcome by causing either "priming" or immunosuppression. This laboratory has recently shown that sublethal hemorrhage (SLH) induces tolerance (decreases mortality/organ injury) to subsequent LPS challenge via a macrophage mediated mechanism. This tolerance is associated with an altered TNF response. The purpose of this study was to determine if this model of tolerance alters the IL-1 and IL-6 response. Rats underwent conditioning with SLH (mean arterial pressure of 30 mmHg for 15 mins). Intraperitoneal LPS (40 mg/kg) was given 24 hrs later. Serum was collected 2 hrs after SLH, just prior to, and 4 hours after LPS. IL-1 and IL-6 were measured by ELISA. Splenic IL-6 gene expression was assessed by RT-PCR.



Tolerance was associated with an increase in both IL-1 and IL-6 in response to SLH. However, tolerant animals elaborated significantly less IL-1 and IL-6 ($p<0.05$ vs sham) when exposed to LPS. The IL-6 gene expression following LPS was not appreciably different between the two groups suggesting a post-transcriptional mechanism is involved. Since the major source of both IL-1 and IL-6 is the monocyte/macrophage, these data further implicate involvement of a "re-programmed" macrophage in production of the tolerant phenotype.

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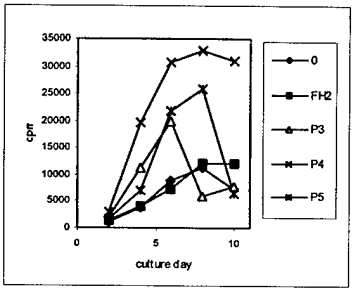
PROLIFERATION OF PERCOLL FRACTIONATED BONE MARROW. G. Noel*, C. K. Ogle, and J. D. Ogle*, Shriners Hospitals for Children, Cincinnati, OH 45229

Colony stimulating factors such as GM-CSF are routinely used to study the production of myeloid cells from bone marrow cells. GM-CSF stimulates myeloid progenitors to divide and mature into macrophages and granulocytes. These progenitors are of low buoyant density and are often partially purified from unfractionated bone marrow cells by a two step Ficoll Hypaque (FH) gradient prior to culture. We have employed a multistep Percoll(P) gradient to further enrich the progenitor cell population. **Methods**: Bone marrow cells from male SD rats (fraction 0) were fractionated on a step gradient of 100,70,60,50, and 40% isotonic Percoll . For comparison, a two step gradient system of Ficoll-Hypaque was used . Fractions obtained from the gradients were numbered in order of decreasing density, with 1 being the heaviest fraction. After fractionation, the bone marrow cells were cultured for 10 days in GM-CSF. Every two days, uptake of ^{3}H-thymidine was measured.

Results: Unfractionated bone marrow (fraction 0) exhibited a steady increase in proliferative rate from days 1 to 8, reaching a maximum of 11,000 cpm. After day 8, proliferation fell slightly. Low density fractions (FH fraction 1, and P fractions 1 and 2) showed no increase over control in proliferation. FH fraction two cells gave a maximum of 47% more proliferation than fraction 0 cells on day 10. In contrast, Percoll fraction 4 showed a 450% increase over control on day 10. Percoll fractionation may be a more efficient method of bone marrow progenitor cell enrichment than the traditional two step gradients.



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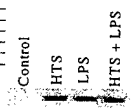
HYPERTONICITY ATTENUATES STIMULATED MACROPHAGE RESPONSES G. Oreopoulos*, I.McGilvray*, S. Rizoli*, Z. Lu*, A. Kapus*, O. Rotstein

Our recent studies have shown pretreatment with hypertonic saline (HTS) in a rodent model of liver ischemia-reperfusion (I-R) has a protective effect during *early* non-neutrophil-mediated injury phases (SIS 1999). Since resident liver macrophages play a role in early hepatic I-R injury, we hypothesized that HTS might exert a beneficial effect by modulating macrophage function. The present studies examine the effects of hypertonic conditions on the stimulated responses of mouse peritoneal macrophages. Thioglycolate elicited peritoneal macrophages harvested from BALB-c mice were treated with isotonic or hypertonic media (500 mOsm) for 2 hours, washed and resuspended in isotonic medium for 4 more hours with or without LPS. Pelleted cells were studied for procoagulant activity (PCA) by one step recalcification and TNF in the supernatants was evaluated by ELISA. Tyrosine and P38 phosphorylation were determined by western blotting. The data suggest that pretreatment with HTS causes inhibition of LPS induced PCA and TNF, an effect which occurs without altered viability as assessed by trypan blue exclusion. The MAP kinase P38 is known to be important in LPS stimulation. To discern whether HTS exerts its effect by inhibition of phosphorylation of P38 cells were evaluated for phospho-P38 after stimulation with LPS (figure), as shown both LPS and HTS induce phosphorylation but HTS does not inhibit LPS induced phosphorylation. Thus: pretreatment with hypertonicity suppresses functional responses of mouse peritoneal macrophages to LPS. This occurs without reducing the signaling pathway leading to P38 phosphorylation. Our data may support the concept that HTS may decrease early hepatic I-R injury by modulating macrophage activity and may be an effective preventive therapy.

Pre-treat	LPS	PCA	TNF
isotonic	-	80.56 +/- 26.50	0.029 +/- 0.068
hypertonic	-	3.38 +/- 3.46	0 +/- 0
isotonic	+	952.74 +/- 314.59 *	3.406 +/- 2.019 *
hypertonic	+	15.89 +/- 22.46 **	0.290 +/- 0.212 ***

*p<0.001 vs. control
**p>0.05 vs. control



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ALTERED MØ IL-15 LEVELS PARALLEL T CELL RESPONSIVENESS POST-TRAUMA. B. Yeh, A. De, K. Kodys, and C. Miller-Graziano. Univ. of Mass. Medical School, Worcester, MA. 01655

Monocyte (MØ) and T cell dysfunctions are well correlated to the development of multiple organ dysfunction syndrome (MODS) after severe trauma. Increased MØ inflammatory cytokine levels, alterations in MØ Antigen Presenting Cell (APC) capacity, as well as T cell immunodepression, are all suggested as contributing to development of MODS. MØ produce, in addition to pro-inflammatory cytokines, monokines that are crucial T cell activators, like IL-12. Another monokine, IL-15, shares many T cell activating properties with the T lymphokine IL-2. MØ IL-15 is also suggested to have unique T cell activation functions in induction of memory T cells. Like IL-12, IL-15 levels increase as MØ differentiate to dendritic cells with augmented APC capacity. Both MØ IL-12 levels and T cell IL-2 levels are depressed in anergic trauma patients. This study assesses the levels of IL-15 mRNA in MØ from normals vs anergic and non-anergic trauma patients to evaluate MØ IL-15 alterations as contributors to post-trauma APC dysfunctions. Methods: Peripheral blood samples are drawn from severe trauma patients (ISS >17, total burn surface area >30%) 2 x/week and assayed parallel to normal controls. mRNA levels from 2 x 10^6 monocytes after stimulation with MDP (20µg/ml) + SEB (500ng/ml) are isolated and mRNA assayed in the Ribonuclease Protection Assay (RPA). Results: mRNA levels of IL-15 are elevated (123% of nor) in MØ from non-anergic trauma patients, but depressed (70%) in MØ of anergic trauma patients. As a trauma patient progresses from T cell responsive to immunosuppressed, their MØ IL-15 levels progress from elevated to depressed. These data suggest that post-trauma MØ dysfunctions consist of selective depression of APC facilitory monokines and augmentation of inflammatory monokines. These selective MØ functional alterations may represent post-trauma differentiation of patient MØ populations toward inflammatory macrophage and decreased MØ to dendritic cell differentiation.

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OPIATE MODULATION OF HEMODYNAMIC, HORMONAL AND TNF RESPONSES TO HEMORRHAGE. N. Ahmed*, N. Abumrad, P.E. Molina. Dept of Surgery, North Shore University Hospital, Manhasset, NY 11030 & Medicine, Brookhaven National Laboratory, Upton, NY 11973.

Studies from our laboratory indicate that CNS-derived efferent pathways which mediate the peripheral metabolic and hormonal responses to stress are modulated by endogenous opiates/opioids. The aim of the present study was to examine the role of endogenous opiates/opioids in modulating the hemodynamic, hormonal and tissue (lung and gut) TNF-α responses to fixed pressure (40 mmHg) hemorrhage (HEM). Chronically catheterized, conscious unrestrained non-heparinized male Sprague-Dawley rats were pre-treated (pre-Rx) with either naltrexone (NAL; 15 mg/kg IP) or saline (0.5 ml) prior to HEM followed by standard resuscitation (Ringer's Lactate). Animals were sacrificed at completion of the resuscitation period. Blood loss required to achieve MABP of 40 mmHg was higher in

NAL pre-Rx animals (4.4±0.2 ml/100 g BW) than in time-matched saline controls (3.7±0.2 ml/100 g BW). HEM increased plasma corticosterone (252±28 to 322±24 ng/ml) and ACTH (106±9 to 387±61 pg/ml) levels within 15 min. NAL pre-Rx prevented the HEM-induced rise in corticosterone, without affecting the rise in ACTH. HEM increased ß-endorphin levels (4-fold) and this was not altered by NAL pre-Rx. HEM produced an immediate (5 min) and progressive increase in circulating epinephrine and norepinephrine levels (to 6801±2702 and 1647±676 pg/ml respectively). NAL pre-Rx did not alter the time course or magnitude of the HEM-induced increase in catecholamines. HEM increased lung and gut TNF content (63% & 25% respectively) over time-matched control values. NAL pre-Rx blunted the hemorrhage-induced rise in lung TNF content and completely abolished the increase in gut TNF. These results indicate that endogenous opiates modulate the hemodynamic instability and hormonal responses associated with HEM. We speculate that as a result, opiates modulate the HEM-induced tissue TNF responses. Supported by ONR Grant # N00014-97-1-0248.

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DECREASED COLLAGEN DEPOSITION AT THE WOUND SITE FOLLOWING HEMORRHAGE: A POTENTIAL MECHANISM FOR IMPAIRED WOUND HEALING. M.K. Angele, M.W. Knöferl*, M.G. Schwacha, A. Ayala, W.G. Cioffi, K.I. Bland*, I.H. Chaudry, Ctr. for Surg. Res. & Dept. of Surg., Brown Univ., Providence, RI 02903.

Several clinical studies indicate that the process of wound healing is impaired in the presence of concurrent trauma, i.e. fracture, thermal injury, or shock. However, it remains unknown whether blood loss *per se* alters wound healing and if so, whether this is due to decreased collagen deposition. To study this, male C3H/HeN mice were subjected to a midline laparotomy (skin and muscle) or a back incision (skin alone) prior to hemorrhage (Hem) (35±5 mmHg for 90 min followed by resuscitation) or sham operation (Sham). At 10 days thereafter, the abdominal wall or the back skin was harvested and the breaking strength (peak load in newton, N) and stiffness of the specimens were determined. Moreover, microscopic sections of the wound site were obtained and stained with Azan-blue for collagen. Additionally, body weight and plasma protein levels were assessed. The results

Peak load abdominal wall (N)		Peak load back skin (N)		Stiffness back skin (N/mm)	
Sham	Hem	Sham	Hem	Sham	Hem
4.2±0.2	2.9±0.5*	4.4±0.3	2.7±0.3*	1.5±0.4	0.6±0.3*

*n=7-8/ group, mean±SEM, t-test, *p<0.05 vs. sham.*

indicate that maximal breaking strength of the abdominal wall and the back incision was significantly decreased in hemorrhaged animals. The stiffness of the back skin specimens was also lower following Hem. Furthermore, decreased collagen staining was evident in the microscopic sections of the wound site following Hem. The impairment of the healing process following Hem does not appear to be due to malnutrition of those animals since no differences in the body weight and plasma protein levels were observed between hemorrhaged and sham animals following wounding. Thus, our data indicates that severe blood loss alters the healing process potentially by decreasing collagen deposition at the wound site. In view of this, attempts to increase collagen production at the wound site in trauma victims might be a useful approach for improving wound healing in those patients. (Supported by NIH GM37127).

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TESTOSTERONE RECEPTOR BLOCKADE ATTENUATES ADRENAL INSUFFICIENCY AFTER TRAUMA-HEMORRHAGE. Z.F. Ba*, P. Wang, D.J. Koo*, W.G. Cioffi, K.I. Bland*, I.H. Chaudry, Brown Univ. Sch. of Med. and Rhode Island Hospital, Providence, RI 02903.

Although studies have shown that testosterone receptor blockade with flutamide restores the depressed immune function and improves cardiovascular and hepatocellular functions in males following trauma and hemorrhagic shock, it remains unknown whether administration of this agent has any salutary effects on adrenal dysfunction in males under such conditions. To study this, male rats (275-325g) underwent laparotomy (i.e., soft tissue trauma) and were bled to and maintained at a BP of 40 mmHg until 40% shed blood volume was returned in the form of Ringer's lactate (RL). They were then resuscitated with four times the volume of shed blood with RL for 60 min. Flutamide (25 mg/kg) or an equivalent volume of the vehicle propanediol was injected subcutaneously 15 min before the end of resuscitation. ACTH (100 µg/rat) induced corticosterone (Cort) release, and adrenal contents of Cort and cAMP, were measured at 20 h after resuscitation. The results were as follows:

	Sham	Hem	Hem + Flu
Plasma Cort (ng/ml)	512 ± 33	370 ± 15*	471 ± 25#
Adrenal Cort (ng/mg)	90.3 ± 7.5	59.3 ± 6.3*	73.7 ± 8.4
Adrenal cAMP (fmol/mg)	1429 ± 54	1025 ± 79*	1309 ± 61#

(Hem + Flu: hemorrhage with flutamide treatment. Data are presented as mean ± SE, n=6/group, and compared by ANOVA and Tukey's test: *P < 0.05 vs. Sham. #P < 0.05 vs. Hem.)

The results indicate that although plasma levels of Cort (non-stimulated) and ACTH in hemorrhaged animals were similar to sham after resuscitation, ACTH-induced Cort, adrenal Cort and adrenal cAMP decreased significantly at 20 h after hemorrhage and resuscitation. Administration of flutamide, however, improved the depressed adrenal Cort contents and restored cAMP, the second messenger of ACTH action, in the adrenal gland after hemorrhagic shock. Furthermore, flutamide improved the diminished adrenal responsiveness to ACTH-stimulation. Thus, flutamide appears to be a novel and useful adjunct for improving adrenal function in males following trauma and hemorrhagic shock (Supported by NIH GM 39519).

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Bile salt supplementation: A new therapeutic approach to interfering with gut-derived endotoxemia?

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Introduction: Intestinal endotoxin has been suggested to play a contributory role in the pathomechanisms of hemorrhagic shock. Bile salts have been considered to bind endotoxin and reduce the intestinal endotoxin absorption. Thus, the objective of this study was to determine effects of bile salts supplementation (BSS) versus bile salts restriction (BSR) on a) fecal endotoxin concentrations, b) plasma endotoxin levels, and six-day-survival rate in rats subjected to hemorrhagic shock. **Materials and Methods:** Animals were pretreated for five days once a day either with a bile salt preparation 1g/kg body weight (BSS group) or saline (CON group). The third group underwent bile duct ligature (BSR group) five days before intestinal endotoxin measurements or hemorrhage (30-35 mmHg, 3h) and resuscitation (1h). **Results:** After five days pretreatment fecal endotoxin levels were significantly increased in BSR animals compared with CON or BSS animals (125.583 ± 4.989 vs. 6.456 ± 1.459 or 6.462 ± 2.473 EU/mg). Three hours after hemorrhage plasma endotoxin levels were significantly higher in BSR animals compared with CON or BSS animals (1.551 ± 0.591 vs. 0.284 ± 0.098 or 0.121 ± 0.027 EU/ml). Also the survival rate after hemorrhagic shock differed significantly between BSR animals and CON or BSS animals, respectively (8% vs. 40% or 73%). **Conclusion:** Bile salt supplementation might provide a new therapeutic approach to interfering with gut-derived endotoxemia.

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RESUSCITATION WITH PACKED RED BLOOD CELLS ATTENUATES HEMODYNAMIC RECOVERY COMPARED TO WHOLE BLOOD FOLLOWING HEMORRHAGIC SHOCK IN CANINES. R.W. Barbee, J.A. Kline and J.A. Watts, Carolinas Medical Center, Charlotte, NC 28232

We assessed if resuscitation from severe hemorrhagic shock with packed red blood cells (PRBC) allows hemodynamic support comparable to that observed with whole blood (WB). Isoflurane anesthetized, non-heparinized canines were instrumented to record mean arterial pressure (MAP, mm Hg), cardiac output (CO, L/min) myocardial oxygen consumption (ml/min/100 g) and left ventricular end-systolic elastance (Ees, mm Hg/ml). There were no significant differences at baseline. Dogs were hemorrhaged to a MAP of 35 mm Hg for 90 min. or until arterial lactate levels reached 7 mM. Animals were first resuscitated with a volume of Ringer's equivalent to two-thirds of the shed blood volume and then an equivalent amount of either WB, or PRBC diluted to an equivalent WB volume with Ringer's (n = 8/group). Animals continued to receive Ringer's at 1 ml/kg/hr to balance insensible fluid loss. Hemodynamic data were assessed at 2 hrs following the onset of resuscitation.

Variable	WB	PRBC
MAP	82 ± 3	72 ± 4*
CO	2.9 ± 0.3	1.8 ± 0.2*
LV dP/dt	1486 ± 126	959 ± 66*
LV -dP/dt	1296 ± 81	944 ± 43*
Myocardial Eff.	30 ± 5	14 ± 3*
Myocardial Work	239 ± 20	128 ± 13*
MVO2	8.9 ± 2.3	6.4 ± 1.2
LV Ees (mm Hg/ml)	6.6± 1.2	4.8 ± 0.8

Myocardial Efficiency (l x mmHg/ml/100g tissue), Myocardial Work (CO X MAP); * = significantly different from WB ($p < 0.05$, unpaired t –test)

A model of resuscitation from hemorrhagic shock with PRBC results in poor hemodynamic recovery compared to that seen with classical WB resuscitation. This study raises concerns regarding the use of PRBC for resuscitation.

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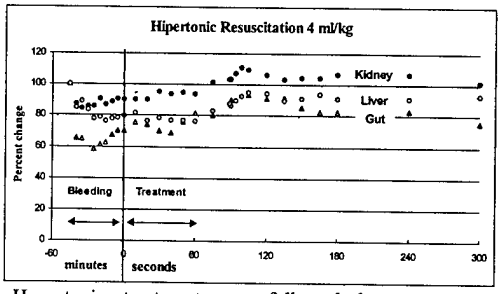
REGIONAL BLOOD FLOW AFTER HEMORRHAGIC SHOCK RESUSCITATION IN DOGS: HYPERTONIC NaCl-DEXTRAN VERSUS ISOTONIC NaCl SOLUTIONS. LFM Barros*, IJ Coelho*, M Rocha e Silva. Heart Institute, State University of São Paulo, São Paulo, Brazil, PO BOX 11450.

Anesthetized splenectomized dogs were subjected pressure driven hemorrhage for 15 min. to reduce mean arterial pressure to 40 mmHg. Thirty min. later, dogs were resuscitated with 32 ml/kg of isotonic saline (n=5), or 4 ml/kg of 7,5% NaCl-6% Dextran (n=6), in 1 min. Before the start of hemorrhage 99m-Technetium labeled erythrocytes were injected in the blood stream, Pre-defined abdominal areas were mapped with a planar gamma-camera. Radiometric readings were corrected for the hemodilution elicited by hemorrhage and fluid resuscitation, based on observed hematocrit changes. Blood flow to the left kidney, liver and gut was estimated through the radiation counts changes observed in the pre-defined organ areas. Images and counts were performed before, during and after hemorrhage (till 30-min post resuscitation). Data are expressed as fractions of the initial value.



Hypertonic treatment was followed by a complete restoration of blood flow to kidney, liver and gut, while the effect of the isotonic resuscitation was only partial. The effect of hypertonic saline was also a longer lasting.

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DIFFERENTIAL EXPRESSION OF VASCULAR STRESS GENES IN HEPATOCELLULAR INJURY INDUCED BY HEMORRHAGE AND RESUSCITATION (H/R) FOLLOWING LIVER CIRRHOSIS. I Bauer*, N Sonin*, X Bian*, J Nicholls*, R Baveja*, Y Yokoyama*, M Bauer, M Clemens, and J Zhang*. Department of Biology, Univ. North Carolina at Charlotte, Charlotte, NC 28223

In this study, we investigated expression of vascular stress genes, endothelin-1 (ET-1) and its receptors (ET_A and ET_B), heme oxygenase-1 (HO-1) and inducible nitric oxide synthase (iNOS) in rat liver cirrhosis and the responses of those genes to H/R. Liver cirrhosis was induced in male Sprague-Dawley rats by common bile duct ligation (BDL) for 7 days. The cirrhotic and sham operated rats were then subjected to hemorrhage (MAP=40 mmHg) for 60 min followed by resuscitation with 60% shed blood and saline for 3 hrs. Blood samples were taken at baseline and 3 hr of resuscitation for enzymatic measurements of ALT and LDH. Livers were harvested before or following H/R and steady state levels of mRNA were estimated by semi-quantitative RT-PCR. Cirrhosis resulted in a significant hepatocellular injury evidenced by elevation in baseline plasma LDH (70±3 U/L, mean±SE vs. sham 45±6 U/L) and ALT (119±14 vs. 37±6 U/L, BDL vs. Sham). H/R exacerbated the injury with a 1298% increase over baseline in LDH compared to a 493% increase for Sham. Changes in ET-1 mRNA paralleled the enzyme release. ET-1 mRNA of BDL rats increased to 336% of Sham and was further upregulated by H/R to 381% of Sham, which was significantly greater than H/R induced increase in Sham (307% of baseline). HO-1 and iNOS mRNA in both Sham and BDL groups showed comparable changes following H/R (see Table). These data demonstrate an upregulation of both constrictive and vasodilatory forces in cirrhotic livers following H/R with a greater expression of constrictor ET-1. The differential expression of these vascular stress genes may ultimately contribute to hepatic microvascular dysfunction and liver failure. *Supported by UNCC Faculty Grant.*

Gene/Group (arbitrary units)	Sham-base	BDL-base	Sham-Hem	BDL-Hem
ET-1	2.18±0.22	7.34±0.06*	6.67±0.15$	8.30±0.45*$
ET_A	9.48±0.52	9.45±0.35	8.40±0.25	8.42±0.64
ET_B	6.08±0.2	7.59±0.87	8.14±0.84	8.68±0.68
iNOS	0	0	6.11±1.96$	4.78±2.07$
HO-1	6.62±0.65	7.59±0.37	8.92±0.53	8.28±0.68

* $p<0.05$ vs. Sham; $ $p<0.05$ vs. Baseline.

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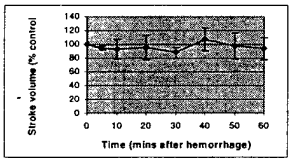
VIDEOMICROSCOPY OF RAT PRE-NODAL MESENTERIC LYMPHATIC VESSELS AFTER HEMORRHAGE B.Boulanger, M.Johnston, J. Ochoa, W.Arden Department of Surgery, University of Kentucky Medical Center, Lexington, Kentucky 40536

Lymphatic vessels may play a dynamic role in blood volume restitution after hemorrhage. Our hypothesis was that pre-nodal lymphatic vessels respond in an effort to

maintain the centripetal flow of interstitial fluid after blood loss. Rats were prepared with arterial and venous catheters and the small intestinal mesentery was exteriorized and placed in Kreb's solution (pH 7.4) for videomicroscopy. Pre-nodal mesenteric lymphatic vessels were identified (50-100 um dia.) and mean blood pressure (MAP), lymphatic contraction frequency (CF) and lymphatic end-diastolic and systolic volumes were measured for 30 mins. Each rat was then bled 1ml/100g body weight over 1 min and observed for 60 mins. after hemorrhage. Lymphatic vessel stroke volume (SV), ejection fraction (EF) and output (Q) were calculated. MAP decreased to 50.9+1.1 mmHg at 5 mins after hem ($p<0.01$) and had returned to baseline at 60 mins. The SV of the lymphatic vessels did not change after



hemorrhage with EF remaining relatively constant. CF varied immediately after hemorrhage but by 60 mins was 60-80% of pre-bleed frequencies ($p<0.05$). Lymphatic vessel Q decreased to 60-80% of pre-bleed levels ($p<0.05$). In conclusion, pre-nodal mesenteric lymphatic vessels in rats maintained their stroke volume and ejection fraction after hemorrhage. These observations support the concept that hemorrhage elicits an adaptive response in lymphatic vessels aimed at the restitution of blood volume.

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LACTATED RINGER'S SOLUTION (LR) EXACERBATES INCREASED MINUTE CARDIAC WORK AFTER DIASPIRIN CROSSLINKED HEMOGLOBIN (DCLHb)

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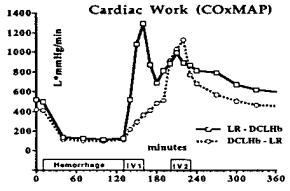
Introduction: Trauma patients resuscitated with blood substitutes often receive large quantities of lactated Ringer's (LR) as well. No data describe the hemodynamic interactions between nitric oxide scavenging DCLHb and LR. **Methods**: Conscious ewes were hemorrhaged for 2 hr, mean arterial pressure (MAP) of 50 mmHg, and randomized into two 30 min infusion groups (n=6). One group received sequential 20 ml/kg DCLHb, followed by 120 ml/kg of LR with 20 min between infusions, the other group (n=6) received identical infusions in reverse sequence. **Results**: MAP increased over baseline with the first infusion of either solution and then decreased rapidly to baseline values after LR but not after DCLHb. Minute cardiac work was only mildly elevated by DCLHb, but LR caused large increases over baseline alone or after DCLHb. The endocardial vitality ratio, an index of oxygen supply/demand, did not decrease under 0.7 when DCLHb was infused first, but it did decrease when LR was infused either after Hb or before Hb. **Conclusion:** Rapid infusion of large volume LR markedly increases minute cardiac work (mainly through increased cardiac output) above baseline levels and exacerbates increases in cardiac work after DCLHb (mainly through increased MAP). Large volume infusion of LR may impair the heart's cardiac oxygen supply/demand ratio. Research supported by Baxter Healthcare.



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EFFECTS OF OXYGEN ON LEFT VENTRICULAR PERFORMANCE IN HEMORRHAGIC SHOCK.

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We have shown that in hemorrhagic shock hyperoxia induces a fast increase in blood pressure and redistribution of blood flow. To clarify mechanisms involved in the rapid hemodynamic response to hyperoxia we evaluated the effects of oxygen on left ventricular performance after hemorrhage. Rats were subjected to circulatory shock by bleeding 30% of their blood volume within 90 min. A left ventricular catheter inserted through the carotid artery and a Heart Performance Analyzer (Micro-med, USA) were used for on-line monitoring of heart rate (HR), end diastolic pressure (EDP), peak systolic pressure (PSP), Maximum dP/dt during contraction and relaxation (MAXdP/dt and MAX-dP/dt) and ventricular pressures at MAXdP/dt and MAX-dP/dt. Inhalation of 100% oxygen was started 20 min. after the bleeding. Data were analyzed by repeated measures ANOVA. Hemorrhage decreased HR and all left ventricular performance parameters ($p<0.01$ from sham rats and from initial values). Inhalation of oxygen did not change EDP and induced an increase in HR ($p<0.01$). In hemorrhaged rats hyperoxia also increased PSP, MAXdP/dt, MAX-dP/dt and ventricular pressures at MAXdP/dt and MAX-dP/dt ($p<0.05$ or less). The relative changes induced by oxygen in HR, PSP, MAXdP/dt, and MAX-dP/dt were significantly higher in hemorrhaged than in sham shock rats. All changes in heart performance parameters returned to control values after cessation of oxygen. Our data suggest that hyperoxia exerts beneficial effects on left ventricular performance in hemorrhagic shock.

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PROTECTIVE ACTIONS OF SODIUM HYDROGEN EXCHANGE INHIBITOR HOE 642 IN A RAT MODEL OF HEMORRHAGIC SHOCK,

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Tissue injury in hemorrhagic shock is mediated by radical and cytokine generation, protease release, and increased neutrophil infiltration. Finally, calcium overload is responsible for irreversible tissue injury.

In the present study we tested the effect of the sodium hydrogen exchange inhibitor HOE642 in a rat model of hemorrhagic shock. Anesthetized rats, subjected to withdraw of blood and reinfusion, developed a severe hemorrhagic shock with marked hypotension, a survival time of 58±9min, and significant increase of intestinal tissue myeloperoxidase ($p\leq0.05$). In histological analyses increased neutrophil infiltration and severe tissue injury was observed. Treatment with sodium hydrogen exchange inhibitor HOE 642 (1 mg/kg) 5min prior to reinfusion of blood resulted in a significant increase of survival time (104±6min, $p<0.05$). Further, administration of HOE 642 inhibited neutrophil infiltration indicated by significantly lower intestinal myeloperoxidase activity in drug treated animals. These results were confirmed in histological sections of HOE 642 treated animals. In addition, a marked reduction of tissue injury could be observed in the kidney, lung, and liver.

Our results indicate that HOE 642 exerts beneficial effects in hemorrhagic shock, thus improving survival and attenuating neutrophil infiltration. Therefore, reduction of calcium overload with the sodium hydrogen exchange inhibitor HOE 642 seems to be a novel strategy to reduce tissue injury in hemorrhagic shock.

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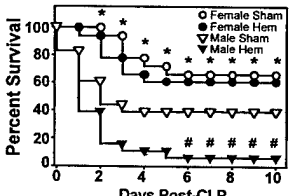
FEMALES TOLERATE THE DELETERIOUS CONSEQUENCES OF HEMORRHAGE AND SUBSEQUENT SEPSIS BETTER THAN MALES.

M.D. Diodato*, M.W. Knöferl*, M.K. Angele, M.G. Schwacha, W.G. Cioffi, K.I. Bland*, I.H. Chaudry, Ctr. for Surgical Research, Dept. of Surgery, Brown University and Rhode Island, Hospital, Providence, RI, 02903.

Although studies have shown that cell mediated immunity is markedly depressed in young males and is enhanced in proestrus females following hemorrhage (Hem), it remains unknown whether this sexually dimorphic immune response to Hem provides any protection against subsequent sepsis. To study this, male and proestrus female C3H/HeN mice were subjected to Hem (35± ± 5 mmHg for 90 min followed by fluid resuscitation) or sham operation. Twenty-four hrs later, all mice were subjected to polymicrobial sepsis by cecal ligation and puncture (CLP) and survival assessed over a 10 day period. The results demonstrated that proestrus females, with cycle increased levels of estrogen, did not display an increased mortality from sepsis following hemorrhage as males did (see figure). In a second group of mice, using the same two-hit model of Hem-CLP, plasma levels of IL-6 and TNF-α were determined at 5 hrs after the induction of CLP. Plasma levels of pro-inflammatory cytokines following Hem-CLP were markedly increased in males, but not in females. Female sex hormones, therefore, appear to play an important role not only in maintaining immunocompetence following Hem, but also in preventing increased mortality from subsequent sepsis. Thus, the adjunct use of female sex steroids/agonists following Hem may represent a novel and safe therapeutic modality for maintaining immune responses and preventing the increased susceptibility to sepsis following trauma. (Supported by NIH GM 37127)



*N=18/group, Z-test, * p<0.05 vs. male hem. #p<0.05 vs. male sham.*

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COMPARISON BETWEEN LACTATED RINGER'S (LR) AND HYPERTONIC ACETATE DEXTRAN (HAD) RESUSCITATION ON TISSUE ANTIOXIDANT STATUS IN AN UNCONTROLLED HEMORRHAGE MODEL IN CONSCIOUS SHEEP.

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It has been proposed that reactive oxygen species contribute to reperfusion injury that may occur after resuscitation from hemorrhage. The present study continues our evaluation of the efficacy of HAD for the treatment of hemorrhagic hypotension. Conscious sheep were subjected to an aortotomy hemorrhage. After 20 min, animals were resuscitated with HAD or LR over 10 min to deliver an equal Na load. A 3rd group received no fluid resuscitation. Animals were euthanitized 4 hr after hemorrhage and tissues collected. Untreated (U) hemorrhaged sheep had significantly lower glutathione concentrations (nmol/mg protein) in lung, ileum and pancreas compared with the LR and HAD groups ($p<0.05$). Indices of lung total antioxidant status were about 30% lower in U than LR and HAD sheep (63±5 vs 85±7 and 93±9 nmol/mg protein, respectively). In addition, catalase and Cu,Zn superoxide dismutase activities (U/mg protein) were significantly lower ($p<0.05$) in lung, ileum and kidney from U animals than LR or HAD resuscitated sheep. Glutathione peroxidase and glutathione reductase activities in kidney from U sheep were about 18% lower than in LR or HAD animals. These data support the observation that hemorrhage is associated with an oxidative stress and this effect was ameliorated to a degree by fluid resuscitation. In the present study, HAD offered a fluid advantage over LR in that 4 ml/kg resulted in equal hemodynamic improvement as 33 ml/kg of LR. HAD, however, showed no special advantage over LR in protecting tissue from oxidative stress.

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INCREASES IN ALVEOLAR MACROPHAGE INTRACELLULAR CALCIUM POTENTIATED BY HEMORRHAGE AND RESUSCITATION

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Introduction: Numerous recent studies have demonstrated that intracellular calcium is elevated after certain types of shock and stress related injury . In addition, evidence supports the contention that increases in intracellular calcium may contribute to cellular dysfunction after injury. The purpose of this study was to define the effect of hemorrhage and resuscitation on alveolar macrophage intracellular calcium.

Methods: Male Sprague-Dawley rats (400-450g) were randomized into four groups: control (non-instrumented), sham (instrumented/non-hemorrhaged), hemorrhage/resuscitated (H/R), and hemorrhage/non-resuscitated (H/NR). Instrumented animals underwent carotid arterial cannulation at time 0 hours. At 24 hours, hemorrhaged animals were subjected to a 25 ml/kg hemorrhage, and resuscitated, if appropriate, after 120 minutes with 50 ml/kg lactated ringers solution. All animals were sacrificed at 48 hours and alveolar macrophages harvested by bronchoalveolar lavage. After harvest, alveolar macrophages were suspended in PBS at $5x10^5$ cells/ml and loaded with 1μM Fura-2. Fura-2 loaded cells were lysed in calcium free buffer and solutions excited at differential wavelengths of 340 and 308 nm. Intracellular calcium concentrations were calculated from Fura-2 ratios.

Results:

	Control	Sham	H / R	H / NR
Calcium (nM)	97.6 ± 7.3	106.5 ± 4.2	152.6 ± 15.3 *	181.5 ± 9.3 *

*p<0.01 vs. Control

Conclusion: These data indicate that alveolar macrophage intracellular calcium concentrations are increased after hemorrhage and are only partially corrected by resuscitation. As calcium is known to be an important intracellular second messenger, these alterations may contribute to metabolic aberrations responsible for post–injury cellular dysfunction .

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IL-11 SUPPRESSES TNF-α EXPRESSION FOLLOWING HEMORRHAGIC SHOCK IN RATS.

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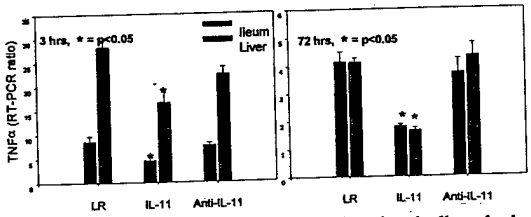
USUHS, Bethesda, MD 20814

Resuscitation following hemorrhagic shock can initiate a cytokine response, and TNF-α has been shown to play an important role in mediating this process. IL–11, an anti-

inflammatory cytokine, may modulate the TNF-α response and improve the outcome in hemorrhagic shock. Methods and Materials. Under sterile conditions, 83 male Sprague-Dawley rats (340-360 grams) were hemorrhaged at 28 ml/ kg body weight over 10 minutes and remained in shock for 75 minutes.The animals were randomized into three groups and resuscitated with 3:1 Lactated Ringer's (LR) solution. The first group received LR only, whereas the other two groups were resuscitated with LR containing IL-11(100 μg/kg body weight) or anti-IL-11(5.7 mg/kg). Forty animals were observed for 72 hour survival and 43 animals were sacrificed at either 3 or 72 hours after hemorrhage to harvest the liver and ileum. Results. IL-11 administration resulted in reeduction of TNF-α specific mRNA levels measured by RT-PCR in ileum and liver of rats at 3 and 72 hours post-hemorrhage as shown below.



TNF-α-positive cells evaluated immunohistochemically at both tissue sites revealed a similar trend. Survival in the IL-11 treated animals (92%) was superior to that observed in animals receiving LR (71%) or anti-IL-11 (78%). Conclusions. IL-11 added to the resuscitation fluid suppressed the expression of TNF-α in tissues and improved survival following hemorrhagic shock in rats.

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L-ARGININE: A UNIQUE AGENT FOR DECREASING LIVER INJURY AFTER TRAUMA-HEMORRHAGE.
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Liver injury is commonly encountered following trauma-hemorrhage (Hem), however, the underlying mechanism is not clear. Although administration of the essential amino acid L-arginine has been reported to restore the depressed cardiovascular functions and cell-mediated immune responses following Hem, it remains unknown whether L-arginine has any protective effects on the liver under those conditions. To study this, adult male rats (n=6-7) underwent laparotomy (i.e., trauma induced), were bled to and maintained at a mean BP of 40 mmHg until 40% of the maximum shed blood volume (MBV) was returned in the form of Ringer's lactate (RL). The rats were then resuscitated (RX) with RL, 4 X the MBV over 1 hr. Sham animals underwent only the surgical procedure. During RX, rats received 300 mg/kg of L-arginine (L-arg) or saline (Veh) i.v.. At 3 and 5 hr after resuscitation rats were killed, blood obtained and the liver fixed for histology (HE staining). Plasma α-GST (GST, ng/ml), a marker of liver damage, and L-arginine (μmol/g protein) levels were assessed. The results

	3 hr			5 hr		
	Sham	Hem	Hem/L-arg	Sham	Hem	Hem/L-arg
GST	61±15	607±73*	268±87*#	64±20	501±99*	107±40#
L-arg	6±0.5	1±0.8*	4±1.5	6±0.4	0.3±0.2*	0.2±0.1*

*Mean±SEM, ANOVA, *p<0.05 vs. sham/Veh, #p<0.05 vs. Hem*

indicate that the increased levels of plasma α-GST in vehicle treated Hem animals were normalized with L-arginine treatment at 5 hr after RX. Moreover, the histology indicated that L-arginine prevented liver edema and neutrophil infiltration following Hem. Plasma L-arginine levels in L-arginine treated rats increased at 3 hr, however, the increase was not sustained and the levels were comparable to vehicle treated animals at 5 hr after RX. Since L-arginine levels decreased rapidly in the plasma following hemorrhage, this amino acid should be considered as safe and inexpensive adjunct to fluid resuscitation, as it not only improves immune and cardiovascular functions but also prevents hepatic injury following Hem. (NIH GM39519).

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DRUGS TO ENHANCE OXYGEN CONSUMPTION IN HEMORRHAGIC SHOCK
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There is a reduction in oxygen consumption during hemorrhagic shock, which is said to correlate with mortality. Although oxygen consumption is often correlated with oxygen delivery, it has also been suggested that the rate of diffusion from the erythrocytes to the mitochondria can be a limiting factor. Two compounds have been found which increase the diffusivity of oxygen through plasma (thought to offer the largest resistance to diffusion): crocetin and a new compound, trans sodium crocetinate (TSC). The addition of either compound to a normal saline resuscitation fluid has been shown to increase oxygen consumption and survival in hemorrhaged rats. TSC is equally effective when infused in smaller volumes. In addition, injections of TSC, rather than an infusion, have also been found to be effective in treating shock. These results are thought to be directly related to the increased diffusivity of oxygen, which has been measured experimentally and verified through computer-based molecular modeling.

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AT-III SUBSTITUTION FOLLOWING MAJOR TRAUMA – IS IT MANDATORY?
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Trauma, major surgery, or septic events may cause relevant reduction in plasma AT-III activity. The latter seems related to outcome. Based on clinical trauma studies, some authors suggest that AT-III substitution may positively affect organ dysfunction as well as outcome. The **aim** of this prospective study was (i) to elucidate alterations in plasma AT-III activity in the earliest period following major trauma and (ii) to assess its relation to IL-6 plasma concentration, an expression of trauma severity. **Methods:** Upon approval of the IRB/EC, 30 patients were enrolled with multiple injuries (ISS Ø 29). Groups were performed according to the peak IL-6 concentration within the first 12 hours (I: <600; II: 600-1200; III: >1200 pg/ml) and survivors vs. nonsurvivors. Blood samples were collected at the scene of accident before primary resuscitation, then every other hour for 24 h. Both AT-III activity and IL-6 levels were determined by commercial test kits. **Results:** All groups revealed a correlation between ISS and peak IL-6 levels at hospital admission (r=.42, p<.01) and 6 hours later (r=.44, p<.01). In all groups a reduction in AT-III activity occurred, that did not fall below 80% in minor injuries. In contrast, AT-III activity of either other group started with a reduced activity (80%) at the site of accident and decreased further to 40% within 1-2 h. Thereafter, the activity re-increased steadily. Severest trauma (III) revealed not only the steepest rise in IL-6 concentration but, surprisingly enough, showed also the fastest recovery of AT-III activity. There was no impact on outcome. **Conclusion**: Following major trauma there is reduction in AT-III activity starting as early as at the site of accident. Severest injuries were associated with very low AT-III activities and a marked IL-6 response. However, when compared to minor injuries restoration of AT-III activity occurred earlier. Finally, we conclude: AT-III substitution (i) might be useful in major trauma if it starts immediately, (ii) is necessary not longer than 4-6 h and (iii) the current IL-6 plasma concentration apparently governs the recovery of AT-III activity.

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EFFECT OF SMALL VOLUME RESUSCITATION (SVR) AND ANTIOXIDANTS ON THE HISTOLOGY AND OXIDATIVE STATUS IN AN HYPOVOLEMIC SHOCK MODEL. J. Hamar, G. Illyés, +W. Schimetta, § U. Brückner. Budapest, Hungary. Linz, Austria. Ulm, Germany.

SVR has been found to be effective in several forms of shock. It was also superior to RL resuscitation in our earlier hemorrhagic-traumatic shock model. Oxygen free radicals are overproduced in shock. In our present series of studies we wanted to see whether SVR completed with additional antioxidant therapy can improve the outcome of resuscitation in the same model.

Shock model: Arterial blood pressure of the anesthetized rat was reduced to and maintained at 35 mm Hg for 90 min. The entire small intestine and the coecum were exteriorized for the same period of time. At the end of injury three types of resuscitations (I, II, and III) were initialized with: I. SVR (7.2 % Na Cl + 10 % HES, 4 ml/kg), II. SVR + Vitamin coctail (vitamins A, C, and D), III. SVR + SOD (10 mg/kg) + Catalase (20 mg/kg), respectively. SVR was completed with fluid therapy wich lasted for two hours. Parameters of the macrocirculation, histology of the heart, lungs, liver, kidney, and the gut, and indicators of the oxidative status (GSH, MPO, MDA, and dienes) in the above organs and also in the plasma were measured. 24-hour survival was also followed. A semiquantitative multiparameter scale as an organ score was developed to evaluate histological changes.

Results: changes of cardiac output, stroke volume, and blood pressure were identical in the 3 groups. Survival: I (4/10), II (5/10), III (5/10). Histology score of all organs equally deteriorated, however, it has improved in the gut and lungs by 24 hours post resuscitation in each group. Plasma GSH was higher in groups II and III at early resuscitation. Diene content of the gut and lungs was reduced in II and III at 24 hours. No major changes were found in the other parameters of the oxidative status.

Conclusion: Effective resuscitation with SVR improves histological status of two important shock organs (gut and lungs). Additional antioxidant therapy with vitamins or enzymes improved slightly parameters of the oxidative status, however, this improvement was not enough to effect beneficially survival or parameters of the circulation.

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THE FEMALE REPRODUCTIVE CYCLE IS AN IMPORTANT VARIABLE IN THE RESPONSE TO TRAUMA-HEMORRHAGE AND RESUSCITATION. D. Jarrar*, P. Wang, W.G. Cioffi, K.I. Bland* and I.H. Chaudry. Brown Univ. and Rhode Island Hospital, Providence, RI 02903.

Studies have shown that immune functions in females are enhanced as opposed to decreased responses in males after hemorrhage. However, it remains unknown if such a sexual dimorphism also exits with regards to hepatocellular and cardiovascular functions following trauma-hemorrhage and resuscitation. To study this, male and female proestrus (PRO) and estrus (EST) Sprague-Dawley rats underwent a 5-cm midline laparotomy (i.e., soft-tissue trauma induced) and were then bled to and maintained at a mean BP of 40 mmHg until 40% of the maximal bleedout volume was returned in the form of Ringer's lactate (RL). Rats were then resuscitated with 4 times the volume of shed blood with RL. At 24 h after resuscitation, cardiac index (CI; ml/min/100g) and hepatocellular function, i.e., the maximum velocity of indocyanine green clearance (V_{max}; mg/kg/min) and the efficiency of the active transport (K_m; mg/kg), were determined using an *in vivo* hemoreflectometer. Plasma testosterone and estradiol levels were measured by RIA. The values (means ± SE; n=6-8/group) were:

	CI	V_{max}	K_m
SHAM ♂	39.6±0.9	1.1±0.3	2.4±0.5
HEM♂	30±1.8*	0.2±0.03*	0.9±0.2*
SHAM ♀EST	40.9±4	1.2±0.25	3.4±0.6
HEM ♀EST	32.4±2.5*	0.2±0.03*	0.77±0.09*
SHAM ♀PRO	39.2±1.5	1.1±0.25	3.4±0.7
HEM ♀PRO	38.3±1.7#	1.0±0.2#	3.07±0.5#

(*P<0.05 vs Sham; #P<0.05 vs Hem-♂/♀EST by ANOVA & Tukey's test.)

The results indicate that cardiovascular and hepatocellular functions were significantly depressed at 24 h after trauma-hemorrhage and resuscitation in male and female estrus animals. Females in the proestrus phase, however, had organ functions following trauma-hemorrhage similar to shams. Plasma estradiol levels were highest in proestrus females (P< 0.05), whereas males had high testosterone and low estradiol levels (P< 0.05). Since a low testosterone/estradiol ratio appears to be beneficial for immune, cardiovascular and hepatocellular functions following trauma-hemorrhage, antagonism of testosterone receptors and/or increase in estradiol levels may be novel approaches for improving organ functions under such conditions. (Supported by NIH GM 39519).

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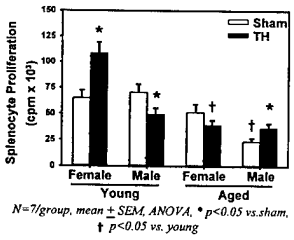
GENDER AND AGE ARE IMPORTANT FACTORS THAT INFLUENCE IMMUNE RESPONSES AFTER TRAUMA-HEMORRHAGE. V. Kahlke*, M.K. Angele, M.G. Schwacha, A. Ayala, W.G. Cioffi, K.I. Bland* and I.H. Chaudry. Ctr. Surgical Research, Dept. of Surgery, Brown University and RI Hospital., Providence, RI 02903.

Recent studies have shown that immune responses are depressed in young males, whereas they are enhanced in young proestrus females following trauma-hemorrhage (TH). Furthermore, young males have increased mortality from sepsis following TH as compared with young proestrus females. Nonetheless, it remains unknown if this sexually dimorphic immune response persists with age. To study this, young (3 mo) and aged (18 mo) male and female CBA/J NIA mice were subjected to laparatomy (trauma) and hemorrhage (35 ± 5 mmHg for 90 min and fluid resuscitation) or sham operation. *In vitro* splenic T-cell responses were determined 24 hrs later. Immune responses were enhanced [i.e., ↑ proliferation (Prolif.), IL-2 & IFN-γ release] in young females following TH, as compared to being depressed in young males (see figure). In contrast, in the aged groups the immune response following TH was reversed (i.e., ↑ Prolif. and IL-2 in aged males and ↓ Prolif. and IFN-γ in aged females). In addition, IL-10 release inversely correlated with age and gender related changes in immune responses following TH, suggesting a role in the immunodepression. Thus, the sexually dimorphic immune response to TH reverses with age. Therefore, approaches to stimulate the immune response in aged females by estrogen replacement and in young males by testosterone receptor antagonism should be considered novel and safe adjuncts to improve immune responses following trauma-hemorrhage. (Supported by NIH GM 37127)



*N=7/group, mean ± SEM, ANOVA, * p<0.05 vs.sham, † p<0.05 vs. young*

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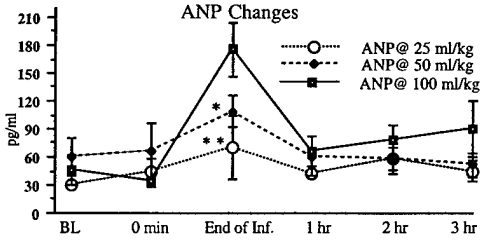
DOES THE DOUBLING OF A RAPID CRYSTALLOID INFUSIONS HAVE ANY EFFECT ON SERUM ELECTROLYTES AND OSMOLALITY IN NORMOVOLEMIC SHEEP ? A.S.Kilicturgay,K.Brauer,D.S.Prough,L.D.Traber,D.L.Traber Department Of Anesthesiology, UTMB and Shriners Burns Institute, Galveston, TX 77555-0833

Introduction: In this study, the effect of the doubling of a rapid crystalloid infusion on serum electrolytes and osmolality was investigated. **Methods**: Four adult female merino sheep (36.5±3.5 kg) were splenectomized and instrumented under halothane in oxygen anesthesia. The sheep were maintained in metabolic cages with free access to food and water and allowed 3-5 days of postoperative recovery. After a 24 hr fast, animals were subjected to three 20 min 0.9% saline infusions in random order: 25 ml/kg, 50 ml/kg, and 100 ml/kg. At least 24 hr was allowed for recovery between infusions. Serum sodium, chloride, glucose, albumin and ANP levels and serum osmolality were measured at baseline, before and at the end of the infusion

and every hour for 3 hours after starting the infusion. Data were analyzed using ANOVA and significance was set at $p<0.05$.

Results: Except ANP, none of these parameters exhibited significant statistical changes among these 3 protocols. Hundred ml/kg of 0.9% saline infused displayed a significant increase in ANP at the end of the infusion when compared with the other two protocols (*p=0.032 and **p=0.008 respectively)



Conclusion: Because of the remarkable doubling of ANP, no significant serum electrolyte or osmolality effect was observed in normovolemic sheep after rapid crystalloid infusion.

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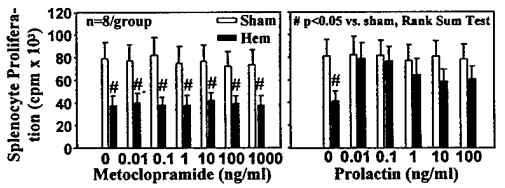
INSIGHTS INTO THE MECHANISM BY WHICH METOCLOPRAMIDE IMPROVES IMMUNE FUNCTIONS FOLLOWING TRAUMA-HEMORRHAGE. M.W. Knöferl*, M.K. Angele, A. Ayala, W.G. Cioffi, I.H. Chaudry, Brown Univ. and Rhode Island Hospital, Providence, RI 02903.

Although studies have shown that prolactin (PRL) as well as metoclopramide (MCP) administration restores the depressed splenocyte immune function following hemorrhage, the underlying mechanism responsible for the immunostimulatory effects of MCP remains unknown. We hypothesized that MCP improves immune responses by upregulating the secretion of PRL. To test this hypothesis, male C3H/HeN mice were subjected to sham operation or laparotomy (i.e., soft tissue trauma) and hemorrhagic shock (35±5 mmHg for 90 min) (Hem) and then resuscitated. Plasma PRL levels were determined 30 min after MCP (1μg/g BW s.c. at end of Hem) or vehicle (Veh) treatment. The results indicate that plasma PRL levels increased significantly in MCP treated mice (sham-Veh 249.9±5.3, Hem-Veh 229.9±7.6, Hem-MCP 596.9±73.1 ng/ml, One way ANOVA, $p<0.05$ vs. Veh). To determine whether MCP produces its salutary effects directly or indirectly via increased PRL secretion, splenocyte proliferation, splenocyte IL-2 and IL-3 release (bioassay) from untreated sham or Hem mice were measured in the presence of increasing concentrations of mouse PRL or MCP.



The addition of MCP had no stimulatory effect on splenocyte functions *in vitro*. However, PRL addition *in vitro* restored the depressed splenocyte proliferation as well as IL-2 and IL-3 release by splenocytes from Hem mice in a dose dependent manner. Thus, the beneficial effects of MCP on immune functions following Hem appear to be mediated by PRL. Since MCP increases plasma levels of the immunoenhancing hormone PRL, MCP should be considered as a useful adjunct for the treatment of immunodepression in trauma victims.

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CRYSTALLOID OR COLLOID RESUSCITATION OF UNCONTROLLED HEMORRHAGIC SHOCK INDUCED BY MODERATE SPLENIC INJURY. MM. Krausz, Y. Bshenko, M. Hirsh, Rambam Med. Ctr., Technion Med. Sch., Haifa, Israel 31096.

We have previously observed that large-volume normal saline (LVNS) infusion in uncontrolled hemorrhagic shock (UCHS) induced by massive splenic injury, leads to increased bleeding and shortened survival, While in moderate splenic injury (MSI) survival was unchanged. In the present investigation we compared the effect of large-volume lactated Ringer's solution (LVRL), hypertonic saline (HTS), and hydroxyethyl starch (HES) in UCHS induced by MSI in rats. The animals were divided into 6 groups: group 1 (n=8) sham operated, group 2 (n=10) MSI untreated, group 3 (n=10) MSI treated by 41.5 mL/kg LVRL, group 4 (n=10) MSI treated by 5mL/kg NaCl 7.5% (HTS), group 5 (n=8) MSI treated by 7.5mL/kg hydroxyethyl starch 6% (HES-7.5), and group 6 (n=8) MSI treated by 15mL/kg HES (HES-15). MSI in group 2 was followed by a fall in mean arterial pressure (MAP) to 77 ±10mmHg ($p<0.001$) in 15 min. A similar fall in MAP was observed in all treated groups. LRVL infusion in group 3 was followed by an early rise in MAP to 97 ±9mmHg ($p<0.01$), which then dropped to 43 ±10mmHg ($p<0.005$) after 60 min. Blood loss in the untreated group was 24 ±5% of blood volume and mean survival time 157 ± 29 min. LVRL infusion resulted in blood loss of 41 ±5% ($p<0.01$) and survival time of 141 ±22 min. HTS infusion was followed by blood loss of 25 ±4% and survival time of 178 ±19 min. HES-7.5 infusion resulted in blood loss of 28 ±6% and survival time of 165 ±19 min., while HES-15 increased blood loss to 47 ±9% ($p<0.01$), but survival time remained 136 ±35 min. It is concluded that LVRL and HES-15 resuscitation in UCHS due to MSI, increase blood loss but do not reduce survival. HTS and HES-7.5 infusion did not alter blood loss or survival.

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EFFECT OF DEHYDROEPIANDROSTERONE ON FUNCTIONAL CHANGES IN KUPFFER CELLS INDUCED BY HEMORRHAGIC SHOCK. J Ku*, CT Hunter*, JP Hunt*, ML Roberts*, CC Baker, and JJ Lemasters*. University of North Carolina School of Medicine, Chapel Hill, NC 27599.

BACKGROUND: Kupffer cells play an important role in the inflammatory response following injury. Previous work showed that hemorrhagic shock induces functional changes in Kupffer cells, which may promote late sequelae such as SIRS and MODS. Dehydroepiandrosterone (DHEA), a naturally occurring steroid hormone intermediate, preserves immune responsiveness during infection and after thermal injury. **AIM**: Using a survivable model of mild hemorrhage, the aim of this study was to test the hypothesis that DHEA modulates changes in Kupffer cells after hemorrhage, possibly preserving a normal phenotype. **METHODS**: After placement of cervical catheters, male Sprague-Dawley rats were administered DHEA (5 mg/kg, s.c.) or vehicle. After 60 minutes, the rats were bled to a systolic blood pressure of 60-70 mm Hg, followed by fluid resuscitation with twice the shed blood volume of lactated Ringers solution. Kupffer cells were then isolated after another 30 minutes. After 48 hours in culture, Kupffer cells were assayed for superoxide production, phagocytosis, and LPS-stimulated formation of tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), nitric oxide (NO), and prostaglandin E_2 (PGE_2). **RESULTS**: Compared to sham-operated, shock caused significant decreases of TNFα (374 ± 119 vs. 1432 ± 241 pg/10^6 cells), NO (5.2 ± 0.9 vs. 11.0 ± 2.1 nmol/10^6) and IL-6 (607 ± 135 vs. 1299 ± 114 pg/10^6). DHEA treatment partially reversed the decrease of TNFα (699 ± 107, p=0.036) but showed a trend to decrease NO further (3.0 ± 1.0, p=0.07). Superoxide and phagocytosis were unaffected by shock and shock/DHEA treatment. **CONCLUSION**: In conclusion, mild hemorrhagic

shock causes decreases in Kupffer cell production of TNFα, NO and IL-6. DHEA restores TNFα production after shock but tends to decrease NO formation further. These changes may be beneficial to the intact organism.

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AMELIORATION BY HUMAN RECOMBINANT P-SELECTIN GLYCOPROTEN LIGAND-1 IMMUNO-GLOBULIN (rPSGL-Ig) OF HEMORRHAGIC SHOCK-INDUCED GUT BARRIER DYSFUNCTION C. Lee*, S. Wattanasirichaigoon*, M. Menconi* and M. Fink. Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215.

Hemorrhagic shock (HS) followed by resuscitation (R) leads to increased intestinal permeability. Increased adhesion between neutrophils and endothelial cells occurs in microvascular beds following HS and may contribute to organ system dysfunction. rPSGL-Ig is a homodimeric molecule which binds P-selectin. We hypothesized that blockade of selectins by rPSGL-Ig can decrease intestinal barrier dysfunction induced by HS. Rats were bled to and maintained at a mean arterial pressure (MAP) of 40 mm Hg. When maximal bleedout (MBO) was achieved, 40% of MBO volume was returned as Ringers' lactate solution (RLS). Either rPSGL-Ig (0.4 mg/kg, *i.v.* bolus and continuous infusion of 0.4 mg/kg/h) or vehicle (n=8/each) was given 1 min prior to R. Thereafter, the animals were resuscitated with four times MBO volume as RLS. Via a midline laparotomy, four segments of small intestine were harvested at baseline (BL), end of shock, and 30 and 60 min after R (R30 and R60, respectively). Intestinal permeability was assessed using an everted gut sac technique, which FITC-dextran (M.W.= 4 kDa) was used as a probe. MAP, blood gas, lactate and intestinal blood flow were not significant difference between the two groups. Conclusions: Treatment of rats with rPSGL-Ig resulted in decreased intestinal barrier dysfunction in a clinically relevant model of HS. rPSGL-Ig warrants further evaluation of therapeutic agent to ameliorate organ injury due to HS and R.

Time	Clearance (nl/min/cm^2)	
	Vehicle	rPSGL-Ig
BL	6.07±1.12	5.72±0.98
Shock	32.03±3.4†	28.69±4.18†
R30	46.95±8.97†	20.74±5.47*†
R60	45.76±8.13†	19.40±6.74*†

Note: *p<0.05: vehicle *vs* rPSGL-Ig and †p<0.05 *vs* baseline within group by ANOVA test.

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MESENTERIC LYMPH FROM RATS SUBJECTED TO HEMORRHAGIC SHOCK IS CYTOTOXIC TO RAT PULMONARY MICROVASCULAR ENDOTHELIAL CELLS. Q. Lu*, D. Xu, C. Adams*, E. Deitch, UMDNJ-New Jersey Med. Sch., Newark, NJ, 07103

We previously have documented that gut-derived lymph from rats subjected to hemorrhagic shock is cytotoxic to human umbilical vein endothelial cells (HUVEC). To verify these results in a rat system, the cytotoxicity of rat mesenteric lymph on rat pulmonary microvascular endothelial cells (RPMVEC) was compared to HUVEC. Methods: RPMVEC isolated from male S-D rat (200g) were grown in 24 well plates. Mesenteric lymph was collected from male S-D rats subjected hemorrhagic or sham shock (30 mmHg for 90 min). Lymph was centrifuged to remove the cellular component, diluted to 10%. Post-shock or sham shock lymph or portal vein plasma collected at sacrifice was incubated with RPMVEC or HUVEC. After various periods of incubation, lactate dehydrogenase activity (LDH) in the cell supernatant was measured by the pyruvate reduction assay and cell viability was determined by trypan blue dye exclusion. Results: The pattern of shock-lymph-induced cytotoxic was similar between RPMVEC and HUVEC. After 6 hrs of incubation, the viability of cells was significantly decreased and LDH activity was increased. Following overnight incubation (16 hrs), the cell monolayers were destroyed. The results are summarized in the following table:

Group	Incubatiion Time	RPMVEC Viability (%)	RPMVEC LDH (U/L)	HUVEC Viability (%)	HUVEC LDH (U/L)
medium		94±0.9	5.4±1.7	91±0.4	7.6±0.7
SK lymph	2h	91±3.1	1.5±0.8	70±1.3	28±11
SK lymph	4h	75±9	4±1.6	63±7[a]	40±11
SK lymph	6h	61±11[b]	12±0.7	48±14[b]	56±12[b]
SK lymph	16h	4±1.2[c]	82.9±14[c]	2.4±1.3[c]	83.2±13[c]
SM lymph	16h	93±3	1.1±0.7	83±0.9	13±2.1
Plasma	16h	94+0.7	5.8+4.5	84+0.9	14+3.6

SK: post shock; SM: sham shock; Plasma: portal vein plasma;
Data are expressed as mean±SE, N=4; [a] p<0.05 vs. medium; [b] p<0.05 vs. medium, sham shock lymph and portal plasma;
[c] p<0.01 vs. all other groups;
Conclusion: These results provide further evidence that post-shock lymph is cytotoxic to endothelial cells and suggest that gut-derived lymph following hemorrhagic shock may contribute to microvascular derangements in distant organs.

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BENEFICIAL EFFECTS OF RU486 ON GLUCOSE-6-PHOSPHATASE GENE EXPRESSION DURING HEMORRHAGE AND RESUSCITATION. Subir R. Maitra, Shiying Wang, Rafaat El-Maghrabi. Trauma Research Lab, Depts of Emerg Med and Surg, Univ Hosp and Med Ctr, SUNY, Stony Brook, NY 11794.

To assess the role of glucocorticoid receptor antagonist and mediator released by Kupffer cells and other resident macrophages, we have used RU486 and gadolinium chloride to prevent the induction of glucose-6-phosphatase (Glu-6-Pase) gene expression in liver following hemorrhagic shock (HS) and lactated Ringer's resuscitation (LR). HS was induced in fasted, anesthetized and cannulated rats by rapid phlebotomy to a mean arterial pressure of 40 mm Hg and maintained for 30 min by withdrawal or infusion of blood. LR group underwent induction and maintenance of HS for 30 min followed by LR resuscitation. Rats were injected with gadolinium chloride (7 mg/kg, Sigma) to inhibit the phagocyte function of Kupffer cells, and with glucocorticoid receptor antagonist RU486 (20 mg/kg) prior to induction of HS. Arterial blood samples were obtained and livers were freeze-clamped in liquid nitrogen and stored at -70°C for subsequent analysis. Northern blot analysis indicated that Glu-6-Pase mRNA abundance increased 2.5 fold in gadolinium chloride pretreated HS rats compared to control. The expression increased further in gadolinium pretreated LR rats. In contrast, mRNA abundance decreased in RU486 pretreated HS rats compared to control. The mRNA abundance returned to normal in RU486 pretreated LR rats. These results indicate that gadolinium chloride was unable to revert the Glu-6-Pase gene expression to normal, whereas, the glucocorticoid receptor antagonist, RU486 was able to prevent the increase in Glu-6-Pase gene expression observed after 30 min of HS and LR rats. (NIH R01 GM52025)

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GASTRIC VS INTESTINAL MUCOSAL REGIONAL PCO_2 ($PrCO_2$) FOLLOWING SHOCK RESUSCITATION: CHANGES WITH SMALL INTESTINAL ENTERAL FEEDING. BA McKinley,* RG Marvin,* FA Moore. Univ of TX-Houston Medical School, Houston TX 77030

Automated tissue CO_2 gas tonometry (Datex-Ohmeda, Helsinki) provides an indirect monitor of gut perfusion and/or mucosal metabolic stress. We compared gastric ($GPrCO_2$) and proximal small intestinal PCO_2 ($IPrCO_2$) after resuscitation from hemorrhagic shock during early

enteral feeding in 9 patients admitted Feb-Aug 1998 (ISS 34±7). After standardized 24-hr resuscitation, including $GPrCO_2$ monitoring, a 2nd tonometry catheter was placed endoscopically in the proximal small intestine during jejunal feeding tube placement. Feeding was advanced by protocol to a caloric goal, with tolerance monitored at 12 hr intervals. 1877 $GPrCO_2$-$IPrCO_2$ data pairs were obtained during 2926 patient ICU hours. Overall correlation r=0.22 ($p<0.05$). The table shows G- and $IPrCO_2$ before feeding, during advancement, while at caloric goal, and when feeding was withheld due to intolerance. (mean±sem; G, $IPrCO_2$[=]mmHg; +$p<0.05$ re pre $IPrCO_2$, *$p<0.05$ re $GPrCO_2$; paired t tests)

JEJUNAL FEEDING	$GPrCO_2$	$IPrCO_2$	n
pre	60±0.7	52±0.4*	383
advancement	58±0.5	61±0.6*+	846
goal	57±0.8	65±1.3*+	113
hold	63±0.5	65±0.7*+	535

Prior to feeding, $GPrCO_2 > IPrCO_2$, but $IPrCO_2$ increased significantly with feeding and during periods of intolerance to reverse this relationship. $GPrCO_2$ and $IPrCO_2$ correlated poorly during all intervals, suggesting differences in gastric-small intestinal mucosal perfusion. Increased $IPrCO_2$ during advancement and target feed rate may indicate perfusion deficit of the intestinal mucosa due to metabolic demands of enteral nutritional support.

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HEMORRHAGE ALTERS TNF RESPONSE TO LPS. P.E. Molina, G. Bagby, N.N. Abumrad. Dept of Surgery, North Shore University Hospital, Manhasset, NY 11030, Medicine BNL, Upton, NY 11973 & Physiology LSUMC, 70112.

Studies suggest that prior exposure to stress alters the cytokine responses to a second insult. The aim of the present study was to determine the effects of fixed pressure (40 mmHg) hemorrhage (HEM) on the TNF-α response to systemic lipopolysacharide (LPS) administration. ***Methods.-*** Chronically catheterized, conscious unrestrained non-heparinized male Sprague-Dawley rats were randomized to either HEM followed by fluid resuscitation with Ringer's lactate (N=12) or sham (N=12) groups. Following 1.5 h after completion of the resuscitation period HEM and sham animals were randomized to receive either LPS (100μg/100 g BW) or an equal volume of saline intravenously. Arterial blood samples were collected at ninety minutes after LPS administration, immediately prior to sacrifice and exsanguination for removal of the lungs. Bronchoalveolar lavage (BAL) was performed and fluid collected for TNF determinations. ***Results.-*** LPS increased plasma TNF (4590±1085 pg/ml) in sham control animals and this was significantly ($p<0.05$) blunted in HEM animals (1204±407 pg/ml). LPS did not alter BAL fluid content of TNF in sham animals. In contrast, intravenous LPS injection resulted in elevated BAL fluid TNF content (35±17 pg/ml) in HEM animals. LPS produced similar increases in lung TNF content in sham and HEM animals (112±14 & 114±12 pg/mg protein respectively). ***Conclusions.-*** These results indicate altered circulating and alveolar TNF responses to systemic LPS administration following HEM. We speculate that the increase in BAL fluid TNF content following systemic LPS administration could be due to increased leakage into the alveolar compartment of TNF from the systemic vasculature or due to access of LPS to the alveolar macrophage population leading to enhanced production and release of TNF. Further studies are required to dissect the mechanisms for blunted circulating LPS and the possibility of alveolar-capillary injury following HEM . Supported by ONR Grant # N00014-97-1-0248.

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DELIBERATE HYPOTENSION PRESERVES OXYGEN DELIVERY IN VITAL END-ORGANS DURING HEMORRHAGIC SHOCK. P. Ng*, R. Miraliakbari*, B.Taterosian*, V. Kim*, J. Philpott*, M. Swanson*, Y. Sun*, R. Lust, and W. Chitwood*. East Carolina Univ. Sch. Med., Department of Surgery, Greenville, NC 27858-4354.

Volume resuscitation represents the standard management for hemorrhagic shock for rapid normalization of blood pressure. Recent studies question this strategy, and instead promote limited resuscitation, minimizing hemodilution, and accepting stable hypotension. This study was designed to assess the efficacy of delayed resuscitation on physiologic autoregulatory mechanisms. Vital end-organ oxygen delivery in severe hemorrhagic shock was evaluated in seventeen anesthetized ventilated mongrel dogs that underwent controlled exsanguination. Dogs were hemorrhaged to a mean arterial blood pressure (MAP) of 40 mm Hg (~40% blood loss), and remained unresuscitated for one hour. Observations included systemic and intracranial pressures (ICP, Camino bolt), blood flow (BF, microspheres, ml/min/100g), arterial blood gas, glucose, and hematocrit (HCT), and oxygen delivery (DO_2). Arterial and sagittal sinus blood samples were drawn at baseline, five minutes after exsanguination, and one hour after exsanguination. The results demonstrated a MAP decrease from 86 ± 14.5 to 45 ± 8.0 (mm Hg, $P<0.05$) which remained stable for the following hour. During the hypotensive period, no significant change in DO_2 occurred in the brain, heart, kidney, liver, or intestine as compared to baseline ($P<0.05$). The lung and pancreas showed the only significant decrease in DO_2. Interestingly, the cerebral oxygen consumption increased despite hypotension in the anesthetized preparation (1.8-3.2 ml/min/100g, $P<0.02$). The HCT, arterial content of oxygen, pH, and alveolar-arterial gradient demonstrated no significant differences. In conclusion, stable hypotension without resuscitation following one hour of severe hemorrhage was associated with an increase in cerebral oxygen consumption and generalized preservation of systemic DO_2 to critical end-organs, suggesting the potential safety of immediate transport with delayed resuscitation

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ACUTE ALCOHOL INGESTION CAUSES IMMUNE MODULATION IN A MOUSE HEMORRHAGE MODEL
D. Oleynikov*, R. Barton, R. Watt*, J. Shelby
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A large percentage of trauma patients are intoxicated and in this population clinical studies suggest an increased incidence of septic complications. The aim of this study was to describe the acute effects of alcohol on the kinetics of inflammatory cytokine production in a mouse model of hemorrhage and survival after LPS challenge. **Methods:** C3H/HEN male mice (n=5/gp) were given 3g/kg/bw of 20% ethanol by oral gavage (BAL-200mg/dl at 90min) followed by hemorrhage to a mean BP of 40mm Hg. for 1hr and sacrificed at 48hrs for spleen harvest. Supernatants from antiCD3 activated spleen cell cultures were assayed by ELISA for IL-6, IL-10 and IFNγ. In a second experiment untouched controls, acutely intoxicated mice (90min post ingestion) and mice 72hrs after alcohol ingestion were injected with LPS (125ug) IP.

Results: Acute ethanol ingestion in hemorrhaged mice attenuated shock-induced increased splenocyte production of IL-6, IL-10 and IFNγ.

Groups	IL-6	IL-10	IFNγ
Control	1556±127	750±192	34810±2665
Shock	3021±498	1191±40	49004±9591
Shock+Etoh	2088±50*	724±70*	29367±1230*#

*$p<0.05$vsShock; #$p<0.05$vsControl (ANOVA) (pg/ml)±SEM

Acute alcohol ingestion was protective against LPS challenge (66% survival) compared to 0% survival in the control and

delayed alcohol (72hrs) groups ($p<0.001$ Kaplan-Meier survival analysis). **Conclusions:** This study demonstrated that acute ingestion of alcohol at physiologic doses attenuated shock-induced increases in inflammatory cytokine production. Alcohol was protective against LPS challenge in acutely intoxicated mice, but offered no protection at LPS challenge 72hrs post ingestion. Alcohol ingestion in this model of shock appears to influence the inflammatory response associated with hemorrhage and LPS challenge, with variable effects depending upon the timing of alcohol ingestion.

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ASSOCIATION BETWEEN CELL-FREE HEMOGLOBIN AND HYPERTONIC SALINE RESULTS IN SINERGYSTIC BENEFITS AS A SMALL VOLUME FORMULATION FOR THE INITIAL MANAGEMENT OF HEMORRHAGIC SHOCK. LF Poli de Figueiredo, EY Varicoda*, JLM Braz*, CB Murta*, M Rocha e Silva. Research Division, Heart Institute-InCor, Univ. São Paulo, SP 05403, Brazil.

Introduction: Hypertonic saline solution (7.5% NaCl, 2400 mOsm/l), infused to hemorrhaged animals, rapidly induces plasma expansion and vasodilatation, restoring cardiac output and arterial pressure. However, its osmotic-driven plasma expansion reduces hematocrit and oxygen-carrying capacity. On the other hand, cell-free hemoglobin efficiently transports oxygen to tissues, but causes limited plasma expansion and vasoconstriction in hemorrhaged animals. We tested the hypothesis that the association of cell-free hemoglobin and 7.5% NaCl produces synergistic benefits in shock. Methods: Pentobarbital anesthetized mongrel dogs (15±1kg) were bled to a mean arterial pressure (MAP) of 40 mmHg in 5 min and maintained at this level for 45 min (shed blood volume = 48±2 ml/kg). Animals were treated with a 4 ml/kg bolus over 4 min of one of the following fluids: whole blood (WB, n=4), 7.5% NaCl (HS, n=5), 13g/dl of glutaraldehyde-polymerized bovine hemoglobin (PBHb, n=7) or 7.5% NaCl/polymerized bovine hemoglobin (HBHS, n=6). No additional intervention was performed and the animals were followed for 60 min after treatment. Results: PBHb and HbHS ($p<0.05$) produced a sustained, significant increase in MAP However, cardiac output was transiently increased only after HS and HbHS ($p<0.05$). Pulmonary arterial pressure remained below baseline. A partial increase in superior mesenteric artery blood flow was observed only after HbHS. Conclusion: Cell-free hemoglobin in small volumes restores MAP in hemorrhagic shock. Its association with hypertonic saline solution resulted in additional improvements in cardiac output and mesenteric blood flow, suggesting a potential benefit for the initial management of major blood loss.

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LOCAL LACTATE & HISTAMINE CHANGES IN SMALL BOWEL CIRCULATION MEASURED BY MICRODIALYSIS IN PIG HEMORRHAGIC SHOCK. D.Rixen[1], H.Goller[1*], H.Wiebe[2*], S.Heß[2*], L.Tuomisto[3*], M.Raum[1*], E.Neugebauer[2], The Shock& Trauma Study Group Dept. of Surg.[1], Biochem.& Exp. Div.[2], II. Dept. of Surg., Univ. of Cologne. 51109 Cologne, Germany; Dept. of Pharm. & Toxic., Univ.Kuopio, SF-70211 Kuopio, Finnland.

Objectives: Hemorrhagic shock results in inadequate organ perfusion & tissue oxygenation. Plasma lactate (L) is a valid parameter to characterize the degree of systemic oxygen debt (OD), but gives no information on local changes. Thus, the aim was to characterize different degrees of hemorrhagic shock by microdialysis measurement of L and histamine (H) in small bowel circulation. Methods: 38 pigs were anesthetized & randomized to one of five groups of increasing OD (<50 to >120 ml/kg). After steady state, the predetermined OD was accrued by hemorrhage uniformly over 60 mins and followed by retransfusion, recovery under anesthesia for 140 mins & observation for 3 days. In parallel to plasma samples, subserosa(*ss*)-, submucosa(*sm*)-, and intraluminal(*il*) L & H probes were obtained by microdialysis at predefined regions of small bowel every 30 mins for 210 min. L was determined by photometry, H by HPLC. Results: Within 60 mins of hemorrhage *ss*- & *sm* L increased from 1.2±.36 & 1.18±.34 to 2.57±.94 & 2.96±1.69mmol/l. Highest mean L>3.5mmol/l resulted 90 & 120 mins after induction of hemorrhage. While *ss*- & *sm* levels hardly differed, *il* L was sign. decreased with 0.27±0.14 mmol/l at 0 mins & highest mean L at 120 mins: 2.45±2.84 mmol/l. *Sm* L was sign. increased after 60, 90, 120 & 150 mins of highest hemorrhage severity (OD>100ml/kg). H dialysates showed no effect either over time nor with the degree of hemorrhage. Highest H was found in steady state after tube insertion and in some animals prior to death. Conclusions: Microdialysis allows a precise evaluation of local L changes in small bowel circulation in pig hemorrhagic shock. *Sm* L levels correlate with the degree of hemorrhagic shock.

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BLOOD LOSS AND TRANSCAPILLARY REFILL IN UNCONTROLLED TREATED HEMORRHAGE IN DOGS. EA Sallum*, S Sinosaki*, FL Pereira*, FY Hondo*, RS Coimbra, PD Branco*, M Rocha e Silva. Research Division, Heart Institute-InCor, Univ. São Paulo, SP 05403, Brazil.

Introduction: The initial treatment of severe blood loss is controversial, regarding the start of volume replacement, the type of solution, the risk of inducing rebleeding after initial treatment and evaluation of transcapillary refill. *Methods*: Pentobarbital anesthetized mongrel dogs (17 ± 2 kg) were used. A portable gamma camera was positioned over the abdomen. Bilateral common iliac artery punctures induced a retroperitoneal hematoma (**RH**). After 30 min, dogs were divided into three groups: untreated (**NT**, n=7), treated with 32mL/kg of Lactated Ringer's solution (**LR**, n=7) and 4mL/kg of 7.5% NaCl plus 6% Dextran 70 (**HSD**, n=7). They were observed for 45 minutes after the start of treatment.

		30 min	75 min	p
NT	red cell loss(%)	31.2	29.3	> 0.95
	refill (ml/kg)	+16.9	+17.3	> 0.95
LR	red cell loss(%)	29.4	56.1	< 0.0001
	refill (ml/kg)	+14.2	-3.3	< 0.001
HSD	red cell loss(%)	32.3	50.1	< 0.01
	refill (ml/kg)	+15.2	+28.5	< 0.05

Results: **RH** reduced red cell volume by 31% at 30 min. Rebleeding in **LR** and **HSD** dogs caused additional losses. Transcapillary refill, 15.4 ml/kg at 30 min, increased after **HSD**, remained unchanged in **NT** dogs, but reversed after **LR**. Significant increases were observed for **LR** and **HSD** dogs ($p<0.001$, against **NT**) for cardiac output, right atrial pressure, mean pulmonary arterial and pulmonary capillary (wedge) pressure during the first fifteen minutes after treatment. However, no difference occurred between the 2 treated groups. **LR** and **HSD** caused significant reductions ($p < 0.001$) in systemic vascular resistance, hematocrit and hemoglobin. *Conclusions*: **LR** and **HSD** induced an initial recovery of relevant hemodynamic and metabolic parameters. Rebleeding occurred in both treated groups, with an effective blood volume loss, through rebleeding plus reversed refill in **LR** dogs, but with an effective volume gain in **HSD** animals.

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iNOS REGULATES HEPATIC PERFUSION AFTER SHOCK AND RESUSCITATION. V.Schuchert*, J.Baust*, M.Schuchert*, A. Peitzman, T.Billiar, B.Harbrecht. Dept. of Surgery, University of Pittsburgh, Pittsburgh, PA 15261

Nitric oxide (NO) plays an important role in the pathophysiology of hepatic dysfunction in shock. We and others have shown that inhibition of constitutive NO synthase (NOS) with nonselective inhibitors increases organ injury in hemorrhagic shock. While there is evidence that inducible NOS (iNOS) is upregulated in shock, its role in local organ perfusion is unknown. We hypothesized that iNOS plays an important regulatory role in hepatic perfusion in trauma and shock. To test our hypothesis, rats were subjected to shock with or without infusion of the selective iNOS inhibitor L-NIL (n=6 per group). Ultrasonic transit time and laser Doppler flowmetry (LDF) were used to measure portal vein (PV) and hepatic microcirculatory blood flow. In addition, iNOS deficient, or knockout mice (iNOS-KO), were subjected to shock and resuscitation (n=6 per group) and hepatic microcirculatory perfusion was measured. Mean arterial pressure did not differ among shock and shock-NIL rats, or among wild type and iNOS-KO mice. In rats, PV flow was not affected by NIL infusion. However, microcirculatory flow was significantly depressed in rats given NIL (Figure 1) and in iNOS-KO mice following resuscitation from shock (Figure 2) ($p<0.05$, ANOVA). These differences were not seen in sham operated animals (n=4 per group). Our results indicate that iNOS plays an important role in the regulation of hepatic perfusion after shock and resuscitation.

Figure 1
Hepatic Microcirculatory Flow in Rats

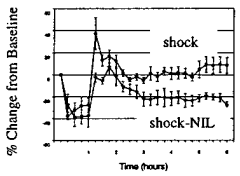
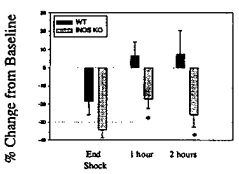


Figure 2
Hepatic Microcirculatory Flow in Mice



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MECHANICAL VENTILATION PRESERVES CARDIAC CONTRACTILITY DURING ABRUPT EXSANGUINATION AND FOLLOWING RESUSCITATION. J.S. Solomon*, M.M. Badellino*, M.W. Grabowski*, S.I.Myers, and R.F.Buckman*. Temple University, Philadelphia, PA. 19140.

Post-hemorrhagic circulatory failure is a common cause of death following injury. The pathophysiology of cardiac dysfunction following abrupt exsanguination is poorly understood and effective therapy remains problematic. This study tested the effect of mechanical ventilation on cardiac function during abrupt exsanguination and resuscitation. Male rats (400-500gm) were sedated with isoflurane. Animals were allowed to spontaneously breathe (SB) or supported with mechanical ventilation (MV). Both groups underwent EKG monitoring and placement of a left ventricular catheter for continuous recording of cardiac mechanics (dP/dt/P). Animals were rapidly exsanguinated (< 2min) via a left femoral catheter to circulatory arrest, defined as pulseless electrical activity (PEA). After three minutes of PEA, shed blood was reinfused. Values for dP/dt/P were averaged over five minute intervals at baseline, immediately prior to PEA, 5 min and 1 hr following resuscitation. Data is expressed as Mean ± SD (N≥7 , *-$p<0.05$ vs MV, by RM ANOVA).

Group	Baseline dP/dt/P (s^{-1})	pre-PEA dP/dt/P (s^{-1})	5 min dP/dt/P (s^{-1})	1 hr dP/dt/P (s^{-1})
SB	262±189	274±126	102±44	202±50
MV	200±69	424±148*	190±67*	301±106

Cardiac contractility during exsanguination increased in ventilated animals when compared to those spontaneously breathing. Furthermore, contractility was higher immediately following resuscitation in mechanically ventilated animals. By one hour this difference was not present but a trend toward higher contractility was noted in the ventilated group. Mechanical ventilation during abrupt exsanguination and resuscitation may preserve cardiac contractility.

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HYPOXIA INHIBITS CELL DIFFERENTIATION X. Song*, J. Wu*, P Rameshwar*, R. Heary*, D. Livingston. UMDNJ-New Jersey Medical School, Newark, NJ 07103

Hemorrhagic shock and hypoxemia have been shown to inhibit hematopoiesis. The mechanisms and location of the defect in differentiation between stem and mature cellar elements remain unknown. We investigated the effect of hypoxia on the differentiation of a well characterized cell line.

METHODS: HL-60 cells were grown in RPMI-1640 with 10% FCS and suspended at a concentration of 1 x 106 cells/ml. Cells were then exposed to hypoxia by placing them in a sealed chamber (95%N2/5%CO2; PaO2 30-35 torr) for two hours. Cells were allowed to recover for 0, 2 or 4 hours and then pulsed with 5% DMSO to induce differentiation. Cells were harvested at 48 hours and stained with nitro blue tetrazolium (NBT). Controls were handled identically, but not exposed to hypoxia. 200 cells were counted per slide and the percentage of NBT (+) cells recorded.

RESULTS: Each time point was done in duplicate and the data for %NBT(+) cells are the mean of two separate experiments. * $p<0.05$ vs. all hypoxic time-points

Normoxia	94%*
Hypoxia + 0 hrs Recovery	7%
Hypoxia + 2 hrs Recovery	19%
Hypoxia + 4 hrs Recovery	23%

CONCLUSIONS: Hypoxia inhibits the differentiation of HL-60 cells into PMN by DMSO. Failure of terminal differentiation may be responsible for hematopoietic failure observed following trauma. Similar to what is observed in patients, this effect appears to be reversible over time.

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THE EFFECTS OF LIMITED RESUSCITATION IN A NEW TRAUMATIC BRAIN INJURY/UNCONTROLLED HEMORRHAGE MODEL. S. Stern*, M. Mertz*, X. Wang*, B. Zink*, S. Dronen*, P Chowanski* (Spon: D. Remick) Univ of Michigan Hlth System, Ann Arbor, MI 48109

Studies of uncontrolled hemorrhage (UH) suggest that initial limited resuscitation (LRES) improves survival. LRES has not been studied in combined traumatic brain injury (TBI) and UH. Objective: To evaluate the effects of LRES on short term mortality and cerebrovascular hemodynamics in a model of combined fluid percussion (FP-TBI) and UH. Methods: 15 anesthetized swine (17-23kg) underwent FP-TBI (3.0 atm) and hemorrhage to a

MAP=30mmHg in the presence of a 3mm aortic tear. Group I(N=7) was initially resuscitated to a MAP=60mmHg, Group II(N=8) to a MAP=80mmHg. After 60 min, the aorta was clamped and animals were resuscitated to baseline physiologic parameters and observed for 150 min or until death. Cerebral blood flow (CBF) was measured using colored microspheres. Results: Mortality was .14 and .50 for Groups I and II respectively (Fisher exact P=.28; 95%CI diff = -.07, .79). Hemorrhage volumes were 40±5 and 69±32ml/kg (t-test P=.02) for Groups I and II respectively. In surviving animals, CPP, CBF, cerebral O_2 extraction ratio (cO_2ER), cerebral O_2 delivery (cO_2del), and cerebral metabolic rate of O_2 ($cmRO_2$) did not differ between groups (repeated measures ANOVA), and at 150 min (after aggressive resusc) did not differ from baseline (paired t-test).

	Min CBF (ml/g/min)	Min cvO_2Sat	Max cO_2ER
Grp I	.655±.165	.63±.08	.36±.08
Grp II	.507±.269	.50±.22	.49±.22
t-test	P=.26	P=.15	P=.10

Conclusion: This is the first combined FP-TBI/UH model. LRES resulted in a trend toward decreased mortality, and was well-tolerated as evidenced by physiologic parameters that remained within the limits of cerebral autoregulation.

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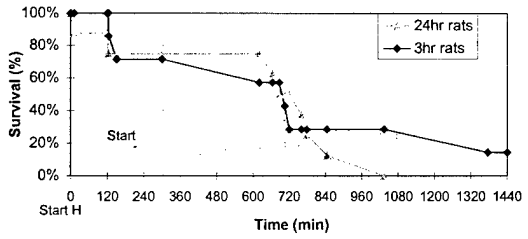
INFLUENCE OF CONTINUED CRYSTALLOID RESUSCITATION ON OUTCOME. A. Tesar,* P. Wall, F. Raymond,* D. Davis,* B. Sobczak,* J. Wittkopf,* T. Ohley,* A. Sidney,* D. Nandal,* A. Chendrasekhar, D. Moorman,* G. Timberlake. Surg Ed & Trauma, IA Methodist Med Ctr, Des Moines, IA 50309.

Fluid resuscitation in hemorrhage models is often short (a few hours). We investigated continued resuscitation with lactated Ringer's (LRS) for 24 hours versus 3 hours. **Methods:** Male Wistar-Furth rats were anesthetized, instrumented, and bled (MAP=35-40mmHg) till MAP≤30 mmHg for 10min or <25mmHg for 1min or 120min elapsed. Resuscitation with LRS (1ml/min) as needed (MAP=75-80mmHg) occurred for 24hr (n=7) or 3hr (n=7). **Results:** Start and two hour resuscitation base excesses were similar (24hr rats, -13.5±1.5 to -5.9±0.6 vs 3hr rats, -16.1±1.8 to -7.3 ±1.8) 24hr rats were bigger (264.6±8.4 vs 219.7±5.0g, p<0.05) and lost less blood (25.4±3.0 vs 37.2±2.7ml/kg, p<0.05). Two hours into resuscitation, hematocrits were 27±2, 24hr rats and 23±2, 3hr rats. 24hr rats received considerably more LRS (521±122 vs 111±26ml/kg, p<0.05), but had no survival advantage.



Conclusions: Continued resuscitation with LRS failed to improve survival after severe hemorrhagic shock. Continuing cardiovascular support with LRS without other interventions is insufficient to improve outcome in this model. (Support: IMMC, VA Med Ctr, ISU, Bayer.)

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ROLE OF ANESTHESIA IN HEART FUNCTION AFTER SEVERE HEMORRHAGIC SHOCK L.R. Thornton, J.A. Kline, J.A. Watts, Carolinas Medical Center, Charlotte, NC 28232

Objective: To test the effect of two in-vivo anesthetic regimens on heart function after severe hemorrhagic shock. Methods: Ketamine/ xylazine-anesthetized or pentobarbital - anesthetized, non-heparanized rats were cannulated via the carotid artery. All underwent tracheostomy. Shocked rats were bled to 25 mmHg for one hour and shams received anesthesia / instrumentation only. Hearts were isolated and perfused in working mode with gassed (95% O_2 / 5% CO_2) buffer containing glucose (G, 11 mM) and were compared to hearts perfused with G (11 mM) + palmitate (P, 0.4 mM). Cardiac efficiency (CE=work / oxygen consumption, where work = cardiac output * peak systolic pressure) were measured by standard techniques. Shock verses control data measured after 30 min perfusion were compared with 3-way ANOVA, n = 5-7 animals per group, *, $p < 0.05$ determined significance.

Results:

		Pentobarbital		Ketamine	
		Control	Shock	Control	Shock
Cardiac Work	G	58 ± 3	59 ± 3	54 ± 2	64 ± 2
	G+P	58 ± 2	48 ± 4*	66 ± 5	66 ± 5
Efficiency	G	1.4 ±.1	1.5 ±.2	1.3 ±.1	1.7±.1
	G+P	1.0 ±.1	0.8 ±.1*	1.7 ±.2	1.4±.2

In the rat, pentobarbital anesthesia plus hemorrhagic shock impaired cardiac function and efficiency, whereas these indexes were preserved with ketamine-xylazine anesthesia plus hemorrhagic shock. These data indicate that ketamine-xylazine is the anesthetic of choice to study the cardiovascular system in rats subjected to hemorrhagic shock.

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TITRATED RESUSCITATION WITH HSD ENHANCES VOLUME SPARING COMPARED TO FIXED VOLUME INFUSIONS

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Small volume resuscitation with hypertonic saline dextran (HSD) is most often administered as a bolus infusion or fixed volume for initial treatment, followed by isotonic fluid. Such studies typically show a 5:1to 10:1 ratio of the volume requirements of isotonic control treatment to HSD treatment, suggesting that equivalent solute loads produce equivalent hemodynamic efficacy. We retrospectively have compared studies in which HSD was administered as a rapid bolus vs studies in which HSD was titrated to physiologic endpoint for resuscitation of hemorrhage and burn shock. Bolus 2 min infusion of 4 ml/kg HSD followed by lactated Ringer's (LR) to treat hemorrhagic shock compared to an isotonic alone group had a 5:1 volume sparing ratio, while using the identical model and HSD titrated to restore and maintain baseline cardiac output resulted in a 14:1 ratio.[1,2] Bolus infusion of 4 ml/kg HSD followed by LR to treat burn shock resulted in volume sparing of less than 2 hrs with no difference after 4 hrs in infused or net fluid.[3] Slow titrated HSD infusion to maintain baseline oxygen delivery after burn shock injury resulted in volume sparing of 73% through 8 hrs postburn of both infused and net fluid balance.[4] This analysis suggests that titrated delivery of HSD increases

efficacy beyond the solute load. Bolus infusions of HSD may cause transient over-resuscitation and be inefficient.

References: 1. *Surgery*, 100: 239–246, 1986; 2. *J Trauma*, 38(4): 602-608, 1995; 3. *J Surg Res*, 50: 272-278, 1991; 4. *Crit Care Med*, 24(11): 1849-57, 1996.

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TIME COURSE OF INDUCIBLE NITRIC OXIDE SYNTHASE AND CYCLOOXYGENASE-2 UPREGULATION DURING HEMORRHAGIC SHOCK

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Both the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are upregulated during hemorrhagic shock (HS) and contribute to organ injury. However, it is unknown when these genes are upregulated following the initiation of shock. Rats were subjected to HS for 1, 2.5, and 5 hours. In separate experiments, after 2.5 hours of HS, rats were resuscitated (R) for 1 and 4 hours. Liver samples were studied for evidence of iNOS and COX-2 mRNA expression using semiquantitative reverse-transcription PCR (n=3 rats for all groups). Comparisons were made with similarly instrumented time-matched sham rats. iNOS and COX-2 expression were detectable as early as 1 hour HS and all further timepoints. During increasing HS duration, iNOS and COX-2 mRNA levels were upregulated 2-4 fold. During increasing duration of resuscitation, iNOS and COX-2 mRNA levels were not upregulated. iNOS and COX-2 expression were upregulated 2-5 fold during R compared with HS. The iNOS inhibitor L-NIL did not inhibit iNOS or COX-2 mRNA expression during HS. The COX-2 inhibitor NS-398 did not inhibit COX-2 or iNOS mRNA expression during HS. iNOS and COX-2 mRNA expression increase early and are upregulated during HS; iNOS derived NO and COX-2 derived prostanoids do not influence COX-2 and iNOS mRNA expression, respectively.

This work was supported by National Institute of Health Grants P50-GM-53789.

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ENALAPRILAT DURING RESUSCITATION INCREASES TEMPERATURE AND IMPROVES SURVIVAL.

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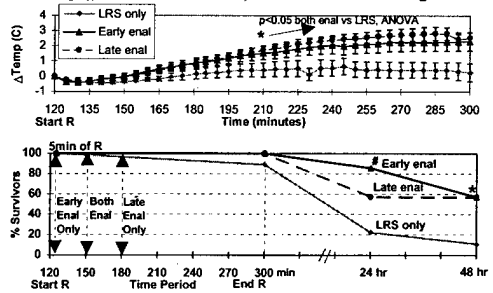
Enalaprilat may improve splanchnic mucosal perfusion and may therefore be useful in resuscitation. **Methods:** Male Wistar-Furth rats were anesthetized, instrumented, and bled (MAP=35-40mmHg) till MAP ≤ 30mmHg for 10 min or <25mmHg for 1 min or 120 min elapsed. Resuscitation with lactated Ringer's (LRS) continued for 3 hours (MAP=75-80mmHg). 9 rats received only LRS (C). 7 rats received enalaprilat (0.06mg/kg) 5 & 30min into resuscitation (Early). 7 rats received enalaprilat 30 & 60min into resuscitation (Late). **Results:** Start resuscitation base excess: C, -12.5 ± 1.1 (standard error); Early, -13.2 ± 0.8; Late, -10.4 ± 0.7. LRS given: C, 72 ± 18ml/kg; Early, 125 ± 24ml/kg (p<0.05 vs others); Late, 64 ± 15ml/kg.



#p=0.008 Early vs LRS. *p=0.05 Early & Late vs LRS. Kaplan-Meier survival curve.

Conclusions: Enalaprilat during resuscitation improved survival, possibly by allowing greater metabolic activity (greater increase in temperature despite room temperature fluids). Delaying enalaprilat administration improved survival but without an increase in fluid requirements. (Support: IMMC, SpaceLabs, Des Moines VA Med Ctr, ISU, Bayer.)

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INHIBITION OF POLY (ADP-RIBOSE) SYNTHETASE DURING RESUSCITATION FROM TRAUMA-HEMORRHAGE IMPROVES VASCULAR CONTRACTILE RESPONSES INDEPENDENT OF iNOS INDUCTION.

J.A. Watts, J. St. John*, R.W. Barbee, N. Sonin*, M.G. Clemens. Carolinas Medical Center, Charlotte, NC, 28232 and University of North Carolina at Charlotte, Charlotte, NC, 28223

These studies examined if inhibition of poly (ADP-ribose) synthetase (PARS) activity prevented the development of vascular hyporeactivity in rats following trauma-hemorrhage and resuscitation. Trauma consisted of a closed laparotomy and rats were hemorrhaged (MAP = 40 mm Hg, 90 min) into a reservoir containing citrate. Resuscitation was 2/3 of shed blood plus 2 1/3 of shed volume as Ringer's lactate. Sham animals received the laparotomy and were time-matched. Induction of iNOS was assessed by RT-PCR. Aortic rings isolated 4.5 hours after resuscitation showed decreased responsiveness to norepinephrine (peak developed tension 0.31 ± 0.01 g/mg tissue) compared with sham rings (0.43 ± 0.02 g/mg) ($p < 0.05$), with no change in EC_{50} for this response (5×10^{-8} M). Addition 3-aminobenzamide (PARS inhibition) at the onset of resuscitation in-vivo prevented decreased responses (0.43 ± 0.03 g/mg). Addition of 3-aminobenzoic acid (no PARS inhibition) did not prevent decreased responses (0.35 ± 0.03 g/mg). These agents did not alter vascular responses in sham animals. iNOS was not induced in the aortas tested. These results indicate that decreased vascular reactivity was prevented by inhibition of PARS and that PARS activation was independent of iNOS induction following trauma-hemorrhage and resuscitation. Support: Charlotte Mecklenburg Health Services Foundation, and NIH DK38201.

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APOPTOTIC CELL DEATH IS INCREASED IN THE BRAINSTEM AFTER HEMORRHAGIC SHOCK IN RATS. JD Weisser, H Laurer, R Raghupathi, and TK McIntosh, Dept. of Neurosurgery, University of Pennsylvania, Philadelphia.

The morphologic correlate for hypotensive episodes in patients who survived hemorrhagic shock is still a matter of speculation. This study was designed to investigate the effect of a fixed-volume hemorrhagic insult on histological changes in the brain. Male Sprague-Dawley rats (300-340g) were anesthetized with sodium pentobarbital (60mg/kg i.p.) and the femoral artery and jugular vein cannulated. The next day the animals were subjected to fixed-volume hemorrhage (8.5ml blood per 300g body weight) within 10 minutes. Animals were monitored every 15 minutes for mean arterial blood pressure (MAP) and heart rate (HR) during the hemorrhage and for 2 hours post injury, and then allowed to recover without blood or fluid resuscitation. Sham animals underwent all procedures except blood withdrawal. Rats were sacrificed by intracardial perfusion with 4% paraformaldehyde on day 1, 3, and 7 after injury, and the brains cut saggitally. Consecutive slides were either stained by cresyl violet (Nissl) or by terminal deoxynucleotidyl transferase mediated dUTP-biotin nick end labeling (TUNEL) and investigated at the light microscopic level. The hemorrhage resulted in a rapid mortality of 30% within the first 30 minutes. During the hemorrhage MAP decreased from baseline level to 40mm Hg, and HR to 40% of baseline. In the following serial measurements, MAP recovered partially to 100mm Hg; nevertheless, the baseline was not reached up to 2 hours. HR increased above baseline and remained elevated. No significant histopathological changes could be detected in Nissl stained slides in any brain region at all time points post injury. However, a significant increase in TUNEL-labeled cells exhibiting apoptotic morphology was observed in the brainstem 1 day post injury. Hemorrhagic shock alone with hypotension of short duration and without resuscitation causes mild pathologic changes in the brainstem. The local character of that finding might be due to an increased vulnerability of cells in the brainstem after severe trauma. Supported by NIGMS R01-GM3690.

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TRAUMA PLASMA SUPPRESSES BONE MARROW PROGENITOR PROLIFERATION THROUGH SOLUBLE MEDIATORS. J. Wu*, P. Rameshwar*, J. Qian , D. Livingston. UMDNJ-New Jersey Medical School, Newark, NJ 07103

Severely injured trauma patients often have decreased bone marrow (BM) functions manifests by persistent anemia and predisposition for infections. Previous work in our lab showed that trauma plasma inhibits the BM progenitors *in vitro*. In this study we attempt to elucidate further the mechanism of this inhibition.

Method: BM stroma was grown in the outer chamber of 24-transwell plates. At confluence, stroma was γ-irradiated with 150 Gy and then incubated overnight. BM mononuclear cells (BMNC, 10^6), from healthy volunteers were placed in the inner wells. Cultures contained 2% v/v plasma from either normal healthy volunteers or trauma patients (n=23). Plasma was obtained from patients admitted to Surgical Intensive Care Unit at various times following injury (12 hours to 28 days). Cultures were incubated for one week after which the BMNC were cultured in short term methylcellulose for myeloid (CFU-GM) and erythroid (BFU-E) progenitors. CFU-GM cultures were supplemented with GM-CSF and BFU-E with IL-3 and erythropoietin). CFU-GM and BFU-E colonies were enumerated after 10 and 14 days respectively.

Results: Colonies with plasma from healthy donors were normalized to 100%. Colonies with trauma patients, represented as a percentage of healthy donors were suppressed for both CFU-GM and BFU-E in the presence of stroma. Also, regardless of the time and injury, trauma plasma was suppressive.

	CFU-GM	BFU-E
w/ stroma	43 ±18 %	40 ±14 %
w/o stroma	106 ± 9 %	49 ±15 %

Conclusion: Direct interactions between BM stroma and progenitors are not necessary suggesting that the inhibitory effects are mediated by soluble factors. The mechanism of inhibition on BFU-E may be direct and indirect.

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HYPERTONIC SALINE AND PENTOXIFYLLINE DIMINISH LUNG INJURY AND BACTERIAL TRANSLOCATION AFTER HEMORRHAGIC SHOCK. MM Yada-Langui*; R Coimbra; CLP Lancellotti*; I Mimica*; C Garcia*; N Correia Jr*, M. Rocha e Silva. Research Division, Heart Institute-InCor, Univ. of Sao Paulo and Dept. of Pathology and Microbiology, Santa Casa School of Medicine, São Paulo, 05403-000, Brazil

Previous reports have shown beneficial effects of Pentoxifylline (PTX) and hypertonic saline (HS) in the treatment of hemorrhagic shock. We compared the effects of these solutions to the conventional lactated Ringer's (LR) treatment on bacterial translocation (BT), lung injury and bronchoalveolar lavage fluid (BAL) after hemorrhagic shock. Rats (280-330g) were bled to a mean arterial pressure of 35 mmHg for 1h and then randomized into 4 groups: LR (3x shed blood); HS (7,5% NaCl, 4ml/kg); LR+PTX (25mg/kg) and SHAM (no shock, no treatment). Additionally, total shed blood was reinfused. At 24 h, animals were sacrificed, aerobic and anaerobic cultures of the liver, mesenteric lymph node complex (MLC), portal blood, and peripheral blood were performed. BT was defined as bacterial growth in the MLC, and bacteremia as growth in the peripheral blood. Lungs were subjected to histopathological examination by a pathologist blinded to the groups, and a score was calculated. BAL was performed at 24h on a separate set of animals that received the same treatments to determine the total (TL) and differential leukocyte counts. Results (mean±SEM).

	HS	LR	PTX
BT(% of animals)	20	80	40
Bacteremia (%)	0	20	0
Score of lung	6.0 ± 1.6	14.8 ± 1.7#	7.8 ± 1.3
BAL (LT)	6.13 ± 1.04	9.2 ± 0.92 ✚	6.3 ± 0.38
% neutrophil(BAL)	5.25	15.8 ⊖	9.72

# $p<0.01$ (LR *vs.* HS, PTX)

✚ $p<0.05$(RL *vs* HS, PTX ⊖$p<0.05$ (RL *vs* HS)

Conclusions: HS and PTX significantly reduced BT and lung injury after shock compared to LR. Lung injury attenuation resulted from less neutrophil infiltration into the lungs of animals treated with HS and PTX.

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EFFECT OF OBSTRUCTIVE JAUNDICE ON VASCULAR RESPONSIVENESS FOLLOWING HEMORRHAGIC SHOCK. RN Younes*, MM Itinoshe*, MM Yada-Langui*, D Birolini*, M Rocha e Silva, Univ. São Paulo LIM-62 and Heart Institute, Hospital AC Camargo, UNIP, São Paulo, BRAZIL

Obstructive jaundice is usually associated with significant hemodynamic alterations, with high rates of post-operative complications. Previous studies showed impaired spontaneous recovery from shock in rats.

Objective: Assess vascular responsiveness following shock in jaundiced animals. **Methods**: Adult Wistar rats (n=20) were randomly assigned to 2 groups: BDL (bile duct ligation jaundiced) or SHAM (sham operation not

jaundiced). A chronic flowprobe was also placed for cardiac output (CO) determination. Seven days following this procedure, the rats were submitted to hemorrhagic shock (MAP=50 mm Hg for 30 min). MAP and CO were continuously registered. The rats were further randomized to receive IV saline (3 ml/kg) or phenilephrine-PHE(0.1 ml/100g) injections, for baseline, posthemorrhage and post30 min shock hemodynamic measurements. **Results**: Table 1 shows the MAP following injections:

Group	MAP baseline	MAP shock
BDL+saline	81 ± 19	55 ± 4
SHAM+saline	89 ± 10	51 ± 1
BDL+PHE	126 ± 22	99 ± 11
SHAM+PHE	126 ± 17	95 ± 7

There was no significant difference between sham operated and chronically jaundiced rats in MAP or CO curves. We concluded that jaundice did not impair vascular responsiveness to phenilephrine in this animal model, either before or after induction of hemorrhagic shock.

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COMPLEMENT ACTIVATION IN HEMORRHAGIC SHOCK. J.Younger*,E.Saleh*,N.Sasaki*,Z.Ravage*,P.Ward, G.Till. Univ.Michigan,Ann Arbor, MI 48109-0303.

The complement system has been implicated in early inflammatory events following hemorrhage. Using a rat model of hemorrhagic shock, we hypothesized that complement would be activated during hemorrhage and that prior depletion of complement would improve hemodynamic response to resuscitation. We also studied if a serum carboxypeptidase inhibitor (SCPNI), which blocks the clearance of C5a, would worsen shock. Rats were hemorrhaged (MAP = 30mmHg) for 1h then resuscitated and observed for an additional 2h. Complement activity was measured at baseline (PRE) and post-injury (POST) by serum hemolytic activity, C3 titer, and C5a ELISA (n=4). To address the 2nd hypothesis, 3 groups of animals [SHAM (n=5), hemorrhagic shock (HEM,n=9), and rats in which complement previously had been depleted with cobra venom factor (HEM+CVF, n=9)] were studied, comparing hemorrhage volume, blood pressure, and metabolic acidosis. To address the 3rd hypothesis, a serum carboxypeptidase N inhibitor was given to SHAM+SCPNI (n=5) and HEM+SCPNI (n=5) animals. Results: Following hemorrhage, serum hemolytic activity was reduced by 59% ($p<0.05$), C3 titers decreased (PRE median=1:640, POST median=1:160, $p<0.05$), and C5a levels increased (PRE=91±58 pg/ml, POST=638±242 pg/ml, $p<0.05$). Complement depletion prior to injury did not affect hemorrhage volume (HEM=28±8, HEM+CVF=28±7 ml/kg, $p=0.88$), but produced trends toward improved final MAP (HEM=47±24, HEM+CVF=67±18 mmHg, $p=0.08$), and final serum bicarbonate (HEM=13±8, HEM+CVF=18±4 mEq/dl, $p=0.12$). Pretreatment with SCPNI produced no effect in the SHAM+SCPNI group, but was associated with death in 80% of the HEM+SCPNI group ($p=0.048$). Conclusion: Complement is consumed and C5a generated during hemorrhage. While improvement in blood pressure or metabolic acidosis could not conclusively be demonstrated following complement depletion, inhibition of serum carboxypeptidase N significantly worsened shock, presumably as a result of impaired C5a clearance.

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CEREBRAL *BCL-2* GENE EXPRESSION AND BLOOD-BRAIN BARRIER FUCTION AFTER HEMORRHAGE AND RESUSCITATION. E. Z. Y. Yu [1], and R. M. McCarron [1,2], (Spon: P. Safar). [1]Naval Medical Research Center, Bethesda, MD 20889; [2]Dept. of Pathology, Uniform. Serv. Univ. Health Sci., Bethesda, MD 20814.

Hemorrhage is a frequent complication of surgery and trauma requiring prompt resuscitation and treatment. The molecular mechanism of the effect of resuscitation must be investigated in order to develop more effective treatment regimen. Recent findings suggest that apoptosis contributes to neuronal damage after ischemic injury. Bcl-2 is an intracellular membrane protein, encoded by *bcl-2* gene, which inhibits apoptosis. P-glycoprotein, a membrane glycoprotein, encoded by *mdr1* (multidrug resistance gene), acts as a pump to transport various cytotoxic agents out of cells and serves as a functional blood-brain barrier (BBB) marker in mammals. This study evaluates the expressions of anti-apoptotic *bcl-2* gene and *mdr1* gene (BBB function) after hemorrhage and resuscitation. Anaesthetized rats were subjected to volume controlled (15 mL/kg) hemorrhage followed by either resuscitation with shed blood (BR, $n = 4$) or non-resuscitation (NR, $n = 4$). Controls had femoral artery cannulation only (SHAM, $n = 4$). Brains were removed 24 h following blood loss. Expressions of *bcl-2* mRNA and *mdr1* mRNA were determined by non-radioactive *in situ* hybridization with self-designed rat *bcl-2* and *mdr1* oligonucleotide probes. *Bcl-2* mRNA levels were upregulated in the cortex, hippocampus, and hypothalamic area of BR group. Lower levels of *bcl-2* gene expression were observed in all three regions in NR rats. *Mdr1* mRNA levels were significantly reduced in the cortex, striatum and hypothalamic area in NR as compared to SHAM animals. However, no or little change of *mdr1* mRNA level was observed in BR group. These findings suggest that blood resuscitation upregulates *bcl-2* gene expression and suppresses apoptosis in response to ischemic injury. Reduced levels of *mdr1* mRNA in NR as compared to SHAM indicate damage to BBB. No change in *mdr1* mRNA levels in BR group suggests protection of BBB function. The data show that blood resuscitation blocks apoptosis and protects BBB function from hemorrhage.

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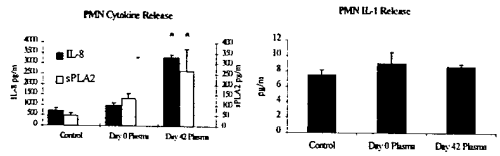
STORED RED BLOOD CELLS SELECTIVELY ACTIVATE PMNs TO RELEASE IL-8 AND $sPLA_2$. G. Zallen*, E. Moore, D. Ciesla*, W. Biffl, P. Offner and C. Silliman*, Denver Health Med. Center, Denver, CO 80204.

PRBC transfusion has been previously invoked with increased infections and immunosuppression, but it has now been demonstrated that stored PRBCs (>14 days) can prime PMNs, and provoke multiple organ failure. Recently, the role of PMNs in the genesis of MOF has been extended to their release of pro-inflammatory cytokines, notably IL-8, secretory phospholipase A_2 ($sPLA_2$), TNF and IL-1. We **hypothesize** that stored PRBCs can act as a second hit and stimulate the release of proinflammatory cytokines from PMNs. **Methods**: Isolated human PMNs were incubated for 24 hrs in RPMI with either 20% fresh plasma or plasma from 42 day old PRBC (day of outdate) and release of IL-8, IL-1, TNF and $sPLA_2$ were measured. **Results**: Plasma from stored PRBCs contained small amounts of IL-8, $sPLA_2$ and TNF 102.1 ±5.6pg/ml, 87.6 ±6.0pg/ml and 9.7 ±.7pg/ml. Levels of IL-1 were below detection (<1pg/ml). Day 42 PRBC plasma stimulated significant PMN release of both IL-8 and $sPLA_2$ as compared to both control and day 0 plasma (*$p<.05$). Day 42 plasma did not stimulate PMN release of either IL-1 or TNF.



Conclusion: Transfused blood is emerging as a pro-inflammatory agent that is capable of producing PMN priming (the first hit). In this study we have demonstrated that PRBC plasma selectively activates PMNs to release both IL-8 and $sPLA_2$ (the second hit). Thus, transfusion of PRBCs may represent a preventable inflammatory insult via modification of both blood banking and transfusion practices.

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GLYCINE IMPROVES SURVIVAL AFTER HEMORRHAGIC SHOCK IN THE RAT. Z. Zhong*, N. Enomoto*, H.D. Connor*, N. Moss*, R.P. Mason*, and R.G. Thurman* (Spon: I. Marzi) Depts. of Pharmacology and Physiology, Univ. of North Carolina, Chapel Hill, NC 27599, and Lab. of Pharmacology and Chemistry, NIEHS, NIH, RTP, NC 27709

This study investigated the effect of glycine on hemorrhagic shock in the rat. Rats were bled to maintain mean arterial pressure at 30-35 mmHg for 1 h and subsequently resuscitated with 60% shed blood and lactated Ringer's solution. Only 20% of rats receiving saline just prior to resuscitation survived 72 h after shock. Survival was increased by glycine (11.2 - 90.0 mg/kg, i.v.) in a dose-dependent manner (half-maximal effect = 25 mg/kg) and reached maximal values of 78% at 45 mg/kg. Eighteen hours after resuscitation, creatinine phosphokinase increased 23-fold, transaminases increased 33-fold and creatinine was elevated 2.4-fold, indicating injury to the heart, liver and kidney, respectively. Pulmonary edema, leukocyte infiltration and hemorrhage were also observed. In the kidney, proximal tubular necrosis, leukocyte infiltration and severe hemorrhage in the outer medullary area occurred in rats receiving saline. Glycine reduced these pathological alterations significantly. It has been reported that oxidative stress and TNF-α production are involved in the pathophysiology of multiple organ injury after shock. In this study, free radical production was increased 4-fold during shock, an effect blocked largely by glycine. Increases in intracellular calcium and production of TNF-α by isolated Kupffer cells stimulated by endotoxin were elevated significantly by hemorrhagic shock, alterations which were totally prevented by glycine. Taken together, it is concluded that glycine reduces organ injury and mortality caused by hemorrhagic shock by preventing free radical production and TNF-α formation by Kupffer cells.

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HYPOXIA, REOXYGENATION AND LPS AFFECT ANTIGEN PRESENTATION AND EFFECTOR FUNCTION IN HUMAN MONOCYTES. H. Bitterman, M.A.Rahat*, M. Ballan*, and N. Lahat*. Carmel Medical Center, Rappaport Institute for Research, Faculty of Medicine, Technion, Haifa 34362, Israel.

Splanchnic ischemia and reperfusion (I/R) and translocation of bacteria or LPS induce systemic inflammatory responses and alterations in immune functions. We simulated I/R in human monocytes in-vitro by exposure to hypoxia/reoxygenation and/or LPS. Experiments were performed in a sealed Plexiglas chamber. Cells were exposed to hypoxia (<1% O_2) for 2 or 24 hours and reoxygenation with air for 2 or 24 hours. Monocytes were double-stained with FITC-conjugated anti-CD14 and PE-conjugated anti-HLA-DR, anti-CD80 or anti-CD86. The percentage of cells expressing both CD14 and each of the molecules was monitored by flow cytometry. Secretion of TNFα was assayed by cytotoxicity in L-929 cells. Hypoxia/reoxygenation and addition of LPS did not change the expression of HLA-DR or CD86 molecules. In contrast, the expression of CD80 molecules, which was observed in 12% of monocytes, was abolished during short and prolonged hypoxia ($p<0.01$) and restored back to normal values only after long (24h) reoxygenation ($p<0.01$). Addition of LPS did not change the suppressive effect of hypoxia or the restoring effect of reoxygenation. Secretion of TNF-α was minimal during short hypoxia and reoxygenation, with or without LPS. Prolonged hypoxia with or without reoxygenation, resulted in a significant increase of TNF-α secretion only in the presence of LPS ($p<0.03$). The data suggest that 1) acute and prolonged hypoxia may reversibly suppress the ability of monocytes to trigger Th1 secreting lymphocytes. 2) Prolonged hypoxia and LPS, synergistically enhance cytotoxic effector functions of monocytes. 3) Impaired antigen presentation seems to precede cytotoxic damage, which largely depends upon LPS. 4) Prolonged reoxygenation normalizes both functions.

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VAGOTOMY BLOCKS THE PROTECTIVE EFFECTS OF I.C.V. CNI-1493 AGAINST LPS-INDUCED SHOCK. L.Borovikova*[1], S.Ivanova*[1], A.Frazier*[1] and K.J.Tracey[1,2]. [1]The Picower Inst. for Med. Res. & [2]Dept. of Surgery, North Shore Univ. Hosp., Manhasset, NY 11030.

The central nervous system plays an important role in the regulation of immune responses, but the mechanism of such regulation is poorly understood. The vagus nerve has been implicated in the interaction between the brain and immune system. Our previous work with the tetravalent guanylhydrazone CNI-1493 indicates that it is a potent central anti-inflammatory agent, but whether these effects are dependent upon the vagus nerve is unknown. Anesthetized vagotomized (VgX) or sham-operated (VgS) Lewis rats were pretreated i.c.v. with saline vehicle (10 μl) as control or with CNI-1493 (1 μg/kg) 60 min before intravenous injection of lipopolysaccharide (LPS) (15 mg/kg). Blood samples were collected at 1 hr after LPS, and the concentration of cytokines (TNF, IL-1β, IL-10) and corticosterone (CORT) measured.

	CNI-1493	TNF, ng/ml	IL-1β, pg/ml	IL-10, ng/ml	CORT, ng/ml
VgS	-	13.8±1.9	88±18	8.22±0.58	850±100
VgS	+	1.2±0.1*	139±52	12.3±1.99*	916±44
VgX	-	18±3.8	88±28	9.04±0.88	570±34
VgX	+	17.5±3.7	90±28	6.36±1.09	670±150

The presented data are mean ± SEM per group of animals (n=5), *- $p < 0.05$ vs zero-dose CNI-1493. LPS induced a significant decrease of mean arterial blood pressure in VgS (65 % of control, $p<0.05$) and VgX rats (65 % of control, $p<0.05$). CNI-1493 completely protected VgS animals against hypotension and blocked TNF production. Bilateral cervical vagotomy reversed the central anti-inflammatory effects, suggesting that i.c.v. CNI-1493 activates vagus nerve dependent signalling pathway to suppress TNF release and attenuate shock.

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T CELL PROLIFERATIVE RESPONSE FOLLOWING SEPSIS IN NEONATAL RATS. O. Dallal,* T. Ravindranath, M. A. Choudhry, A. Kohn,* J. K. Muraskas,* M. M. Sayeed. Departments of Physiology, Surgery, and Pediatrics. Stritch School of Medicine, Loyola University; Maywood, Illinois 60503.

Previous studies in our and others laboratories have shown a decrease in T cell proliferative response during sepsis in adult rats. The present study evaluated whether such proliferative disturbance also occurs during sepsis in 10 and 15 day old neonatal rats. Sepsis was induced in the pups by implanting into their abdominal cavities fecal pellets containing 50 CFU of *E. coli* and *B. fragilis* each. Animals implanted with fecal pellets without bacteria are referred as Sterile. Sterile and Septic pups (24-hour post-implantation) as well as unoperated control pups were sacrificed, and spleens were removed and processed for preparation of single cell suspensions. Cells were counted using trypan blue. T cell proliferative response was measured after stimulation of cells with ConA by quantifying cellular ^3H thymidine uptake. The results are as follows:

	Control (n=8)	Sterile(n=9)	Sepsis (n=13)
Total cell count	$(5.6\pm3.2)10^5$	$(3.1\pm1.7)10^5$	$(3.2\pm1.8)10^5$
Proliferate response (DPM/10^5)	2887±1195	1245±310	894±38

Our data show a decrease in both the total splenocytes count and the proliferative response in the sterile and septic neonatal rats compared with controls.
(Supported by NIH grant GM 53235 and GM 568501).

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CYTOKINE STIMULATION INDUCES PARACRINE REGULATION OF PMN FUNCTION AND APOPTOSIS. P.S. Grutkoski*, R. D'Amico*, A. Ayala and H.H. Simms: Rhode Island Hospital and Brown University, Providence, RI 02903.

Purpose: Cell-cell communication has been demonstrated between PMN and other cell types through the paracrine actions of secreted cytokines. To date, limited data is available addressing PMN-PMN communication. Therefore, the aim of this study was to determine whether PMN were able to affect PMN function *in vitro* in a cell-contact independent manner and whether IL-1ß, IL-8, or TNFα influenced the paracrine effect. Methods: Conditioned medias (CM) were prepared by incubating PMNs in HBSS ± IL-1ß, IL-8, and TNF. PMN were incubated in HBSS or CM and assessed for changes in (1) phagocytosis of FITC-labeled opsonized *E. coli* by FACS (2) apoptosis by morphological examination as well as TUNEL and caspase 3 activity, (3) oxidative metabolism through the activation of dichlorofluorescein diacetate and (4) the cell surface expression several proteins measured by FITC-antibody or radio-labeled antibody detection. Results: Our results demonstrated 1) CM from IL-1ß treated cells (CM-IL1ß) increased phagocytosis 17%, CM-TNF decreased phagocytosis 10%, while CM-IL8 and control CM had no effect. 2) CM-IL1ß and CM-IL8 suppressed apoptosis by 60% and 28%, respectively, when compared to HBSS controls. 3) CM had no effect on oxidative metabolism. 4) CM had no effect on Fc receptors. 5) Effects of CM on the expression of Mac-1, CR1, L-selectin, and PSGL-1 varied between cytokines. Conclusions: PMN are able to communicate with and influence the immunological function and apoptotic activity of other PMNs independent of cell-cell contact. The major impact of this paracrine regulation is the downregulation of PMN apoptosis, demonstrating that the pro-inflammatory cytokines IL-1ß and IL-8 can directly and indirectly inhibit apoptosis. We also show that PMN stimulated with IL-1ß, IL-8, and TNF have the potential to upregulate the PMN inflammatory response in a paracrine manner.

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EVIDENCE FOR FUNCTIONAL ADRENERGIC INNERVATION IN BONE MARROW. S. Jones, R. Shankar,* R. Gamelli and Y. Tang.* Loyola Univ. Med. Center, Maywood, IL 60153

Regulation of proliferation and maturation of leukocytes in the bone marrow has been hypothesized to include the action of sympathetic nerves through the release of norepinephrine (NE). Although there is documentation of measurable bone marrow neurotransmitter NE and histological evidence for bone marrow innervation, functional activation of these nerves to external stimulation has never been demonstrated. The present study assessed the dynamics of NE release in bone marrow in mice in response to well-established protocols known to elevate sympathetic activity. Nerve-stimluated release of NE was estimated using NE turnover techniques invovling either a pulse-chase paradigm of following the fall in NE specific activity [(^{3}HNE)/NE total] or a non-isotopic method of following the fall in tissue NE after inhibition of biosynthesis. Cold exposure (20 hrs at 4°C) increased NE turnover rate in bone marrow by 36% from 0.33 to 0.45 ng/g/hr, while treatment with ganglionic blockade (chlorisondamine) blocked such dynamic NE changes. *Pseudomonas aeruginosa* (administered intraperitoneally, 1-1.5 x10^6 CFU) increased bone marrow NE turnover rate by 131% from 0.13 to 0.30 ng/g/hr. Similar results for infectious challenge were obtained using non-isotopic methods. These results demonstrate that the adrenergic innervation of the bone marrow is functionally dynamic and is responsive to generalized stress. Furthermore these results lend credence to the premise that neural mechanisms may influence the regulation of lympho- and myelopoietic cellular events.
Supported by MH53562 (SJ) and GM56424 (RS).

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THE EFFECTS OF L-ARGININE ENRICHED FEEDING ON THE FUNCTION OF HEPATIC KUPFFER CELLS DURING SEPSIS. Y. Liang*, T.L. Hwang, Department of Surgery, Chang Gung Memorial Hospital, Chang Gung University, Taipei, Taiwan.

Hepatic Kupffer cells are capable of multiple functions, which play an important role in host defense both directly and via the modulation of inflammatory response. Kupffer cells can produce nitric oxide which has cytotoxic effect. The L-arginine-nitric oxide pathway has been studied for years. L-arginine enriched feeding may augment the function of Kupffer cells. Male Spaque-Dawley rats were used. L-arginine (0.5 gm/day) was fed to the rats in Arg. group for 5 days. The rats in Arg. group (n = 6) and control group (n = 6) received caecal ligation and puncture (CLP). Kupffer cells were isolation and cultured. The Kupffer cells function (CINC), TNF, IL-1, IL-6 and nitrite/nitrate were determined and compared. The Kupffer cells from Arg. group can produce higher nitrite/nitrate (16.6 ± 0.7 μm vs 12.2 ± 0.6

μm) ($p < 0.05$), IL-1 (770 ± 71 pg/ml vs 531 ± 44 pg/ml) ($p < 0.05$) and IL-6 (469 ± 32 pg/ml vs 396 ± 92 ng/ml) ($p < 0.05$) than those in control group. They also had significantly higher CINC activity (37.1 ± 3.1 ng/ml vs 15.4 ± 3.7 ng/ml) ($p < 0.05$) than those from control rats. We concluded that the L-arginine enriched feeding can induce better function of Kupffer cells with higher level of nitric oxide, IL-1 and IL-6. The better function of Kupffer cells may be triggered through arginine-nitric oxide pathway.

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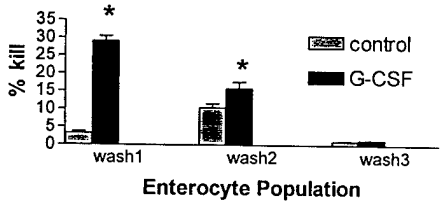
EFFECT OF G-CSF ON ENTEROCYTE BACTERICIDAL FUNCTION Cora K. Ogle, and John F. Valente, University of Cincinnati, Dept of Surgery and Shriners Hospital for Children, Cincinnati Ohio 45229

Intestinal epithelial cells produce bactericidal peptides, termed "cryptdins" as part of their barrier function. The administration of granulocyte-colony stimulating factor (G-CSF) has been shown to confer a variety of expected (and often unexpected) beneficial effects in a number of clinical settings and experimental models. We sought to determine if G-CSF could increase the bactericidal function of isolated enterocytes.

Methods: Recombinant human G-CSF (Amgen) was administered in sterile normal saline at 10 μg/kg subcutaneously for 4 daily doses (N=5) to ACI strain rats 250-350 grams. Animals were then anesthetized and enterocytes were isolated by washing with EDTA 3 times. This method yields 3 populations of enterocytes determined by microscopic examination. For each experiment, sham animals (N=5) receiving only subcutaneous vehicle were used as controls. Bactericidal function of enterocytes was determined by the alamar blue assay using *E. coli.* as the test organism.



* *p<0.05 control vs.G-CSF*

Conclusion: G-CSF can increase the bactericidal function of certain populations of isolated enterocytes.

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EFFECTS OF TUMOR NECROSIS FACTOR-α (TNF-α) AND INTERLEUKIN-1β (IL-1β) ON HUMAN ADRENAL ANDROGEN PRODUCTION. C. R. Parker, Jr*, A. Stankovic *, and M. Jian* , (Spon: S. Reichard). Univ. Alabama at Birmingham, Birmingham AL, 35233.

The immune system is activated and adrenal androgen production is suppressed as part of the physiologic response to many stressors. We sought to determine if factors elaborated as part of the host response to trauma and infections might play a role in the regulation of adrenal androgen production. To this end, we cultured human adrenal cells and investigated the secretion of the major adrenal androgen, dehydroepiandrosterone sulfate (DS) in response to cytokines such as TNF-α and IL-1β. Adrenal cells were cultured for 3 days in the presence of 10, 100, or 1000 pM TNF-α or similar concentrations of IL-1β. Adrenal cells were cultured in 96 well plates in McCoys medium containing antibiotics and antimycotics, and 10% fetal bovine serum. All culture conditions were conducted in replicates of at least 5 wells each, and each experiment was repeated a minimum of 3 times. In all instances, steroidogenesis was stimulated by addition of low concentrations (0.9 nM) of adrenocorticotropin (ACTH). Additionally, we evaluated the effect of combined exposure of human adrenal cells to TNF-α and IL-1β on ACTH stimulated DS secretion. Compared to DS production after 3 days of culture in the presence of ACTH alone, DS production was significantly reduced to 84, 73, and 60% when cultures also contained 10, 100, and 1000 pM TNF-α. Similarly, ACTH stimulated DS secretion was significantly reduced to 62, 54 and 52% of control levels when adrenal cells were co-cultured in medium that contained 10, 100 and 1000 pM IL-1β. When adrenal cells were exposed to ACTH and 100 pM TNF-α, the addition of 10, 100 or 1000 pM IL-1β caused further reductions of DS secretion to 36, 31, and 18% of that seen with ACTH alone. Significant inhibitory effects of these cytokines, alone and in combination, also were seen when added to cells that were co-cultured in the presence of 9nM ACTH. The effects of exposure to both cytokines seemed to be additive rather than synergistic. These data are suggestive that cytokines may play a role in the deficiency of adrenal androgen production associated with many stressors in the human.

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PROLIFERATIVE AND FUNCTIONAL DISTURBANCES IN PERIPHERAL AND MUCOSAL T CELLS FOLLOWING BURN AND BURN-INFECTION. T. Ravindranth, M. Choudhry, S. Namak*, A. Kohn*, A. Kambam*, S. Khan*, X. Ren*, N. Fazal and M. Sayeed. Burn & Shock Trauma Institute and Ronald McDonald Children's Hospital of Loyola Univ. Med. Center. Maywood, IL 60153.

The present study evaluated the proliferative response and IL-2 levels in T-cells of Peyer's patches (PP) and spleen (SP) to understand the gut mucosal and peripheral immune system function relationship after burn (B) and burn infection (BI). These studies were performed in a rat model of full thickness burn, 25% TBSA. Infection was induced by ip injection of *Escherichia coli* (10^9 CFU). The animals were sacrificed 24 to 72 hours following burns. The results obtained were as follows:

Group	$CD3^{\pm}$ (%)		Prolif (DPMx 10^3)		IL-2(ng)	
	PP	SP	PP	SP	PP	SP
C	58±17	28±3	333±10	200±10	4.5±1	4±0.7
CI(d1)	-	-	320±10	180±20	-	-
CI(d2)	51±2	40±4	280±40	120±40	4±1.5	2±0.7
B (d1)	-	-	275±10	175±10	-	-
B(d2)	48±9	30±4	200±20	100±30	1±0.5	0.8±0.2
B(d3)	-	-	40±20	50±30	-	-
BI(d1)	-	-	80±20	55±20	-	-
BI(d2)	45±10	45±10	30±10	40±20	0.6±0.2	0.2±0.3

d 1-3: 1-3 days post-burn; C: Control; CI: Control+infection.

These results indicate that there is diminished proliferative response and IL-2 production in gut mucosal and peripheral T-cells following B and BI. A tendency towards diminished $CD3^+$ cells was also noted in the gut mucosa following B and BI. Our data suggest that the decreased proliferative responses in T cells from PP and SP in B and BI contribute to an overall suppression of systemic cell mediated immunity in the burn as well as burn-infected host. (Supported by NIH GM53235 and GM 568501).

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CHEMOKINE REDUNDANCY ENSURES AN APPROPRIATE BIOLOGIC RESPONSE. D. Remick, L.Green, D. Newcomb, S. Garg, G. Bolgos, and D. Call, U of Michigan, Ann Arbor, MI 48103

Previous publications demonstrated that elevated systemic levels of IL-8 decrease neutrophil (PMN) recruitment to sites of local inflammation. We tested if IL-8 would reduce PMN recruitment to inflammatory insults. Mice transgenic for human IL-8 were separated into IL-8 pos (plasma levels>90 ng/ml) and IL-8 neg (IL-8 below detection). Presence of the IL-8 transgene did not improve survival, alter morbidity or peritoneal PMN recruitment following the cecal ligation and puncture model of sepsis. In an acute lung injury model induced by the intratracheal injection of acid, the IL-8 pos mice had the same degree of alveolar PMN recruitment as the IL-8 neg mice. Additionally, there was no difference in the local recruitment PMN when either thioglycollate (thio) or glycogen was injected intraperitoneally. We examined the chemotactic response of peripheral blood PMN towards murine chemokines. PMN from IL-8 pos and neg mice responded equally well to recombinant KC or MIP-2. We then looked at the local response and within the peritoneal cavity after thio injection the IL-8 pos mice (n=12) had significantly increased levels of the chemokines compared to the IL-8 neg mice (n=13, values are mean ± SEM). Our data demonstrate that even with

	IL-8 pos	IL-8 neg
KC total ng	6.5 ± 2.0	1.8 ± 0.6
MIP-2 total ng	1.9 ± 0.5	0.9 ± 0.4

high plasma levels of IL-8, PMN are still recruited to sites of inflammation, probably by other chemokines. Attempts to reduce PMN accumulation with elevated systemic levels of chemokines may not be effective.

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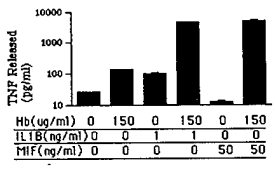
HEMOGLOBIN ENHANCES MIF- AND INTERLEUKIN-1-MEDIATED TNF RELEASE IN MURINE MACROPHAGE-LIKE RAW 264.7 CELLS. **H.Yang, J. Vishnubhakat, H. Wang, J. Roth and K. Tracey.** The Picower Institute for Medical Research and North Shore University Hospital, Manhasset, NY 11030.

Hemoglobin (Hb) enhances endotoxin activity in cell culture and lethality in animal models (Kaca et al., JBC 269:25078-84, 1994). We recently demonstrated that hypophysectomized rats, which had a 2-3 fold increase in serum hemoglobin, are markedly more sensitive to lipopolysaccharide (LPS)-induced toxicity. We also found that hemoglobin, in a dose-dependent fashion, synergized with LPS in enhancing TNF release from RAW 264.7 cells (Bloom et al., Shock 10;395-400, 1998). As these results were restricted to LPS-stimulated macrophages, we sought to investigate whether Hb sensitizes macrophages to other inflammatory stimuli. RAW 264.7 cells (10^5 cells/well, 96 well plate) were treated with human hemoglobin and stimulated with interleukin-1β (IL-1β; mouse recombinant) or macrophage inhibitory factor (MIF) as indicated. TNF was measured by ELISA in supernatants collected 20 hours later.



Our results show that the ability of IL-1 and MIF to stimulate TNF release from macrophage-like cells can be markedly enhanced by Hb at total concentrations similar to those encountered in humans in vivo. As Hb is ubiquitous in patients with burn, injury or trauma, these findings provide a potential mechanism for increased cytokine toxicity in hemoglobinemic patients.

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GRANULOCYTE COLONY-STIMULATING FACTOR SUPPRESSES THE PULMONARY CHEMOKINE RESPONSE IN RATS CHALLENGED WITH INTRATRACHEAL ENDOTOXIN. P. Zhang*, S. Nelson*, W.R. Summer*, G.J. Bagby. Dept. of Medicine - Sec. of Pulmonary/CCM, Dept. of Physiology, and Alcohol Research Center, LSU Medical Center, New Orleans, LA 70112

Granulocyte colony-stimulating factor (G-CSF) augments neutrophil (PMN) recruitment into the lung in response to intratracheal challenges with bacteria or endotoxin (LPS). Relatively little information is known about the effects of G-CSF on pulmonary chemokine profiles during these host responses. This study investigated the effects of G-CSF on pulmonary macrophage inflammatory protein-2 (MIP-2) and cytokine-induced neutrophil chemoattractant (CINC) production in response to LPS challenge. Rats were pretreated for 2 days with a subcutaneous injection of G-CSF at 50 μg/Kg or vehicle twice daily. On the third day, LPS (100 μg) or saline was administered intratracheally 3 hrs after the last dose of G-CSF or vehicle. The animals were sacrificed at either 90 min or 4 hrs after intratracheal challenge. Intratracheal LPS induced a marked increase in MIP-2 and CINC concentrations in bronchoalveolar lavage (BAL) fluid. G-CSF treatment did not alter chemokine levels in BAL fluid at 90 min following intratracheal challenge with LPS (10844±1775 vs. 9905±2559 and 3827±187 vs. 4197±400 pg/ml for MIP-2 and CINC respectively, $p>0.05$). In contrast, LPS-induced MIP-2 and CINC in BAL fluid were significantly decreased (23260±3771 vs. 3487±1145 and 6535± 842 vs. 3277±918 pg/ml respectively, $p<0.05$) in G-CSF-treated rats at 4 hrs after intratracheal challenge. *In vitro*, G-CSF did not have a direct effect on MIP-2 and CINC generation by isolated alveolar macrophages in response to LPS. Pulmonary recruitment of PMN was increased 5 fold (16±5 vs. 81±21 $x10^6$, $p<0.05$) by G-CSF treatment 4 hrs after LPS challenge. These data suggest that modulation of pulmonary chemokine profiles in G-CSF-treated animals is mediated by PMNs. These effects may have beneficial consequences in infected patients with acute lung injury. Supported in part by NIH grant AA09803

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STAPHYLOCOCCUS AUREUS ALPHA TOXIN INDUCES SELECTIN DEPENDENT PMN-INDUCED VASOCONTRACTION AND ENDOTHELIAL DYSFUNCTION, U. Buerke*, U. Sibelius*, U.Grandel*, F. Grimminger*, J. Meyer*, H. Darius*, and M. Buerke, II. Department of Medicine, Johannes Gutenberg University, Mainz, Germany, Department of Medicine II, Justus-Liebig University, Giessen, Germany

Neutrophil adhesion to the vasacular endothelium plays an important role for induction of vasocontraction and endothelial dysfunction.

In the present study we evaluated the effect of the pore forming Staphylococcus aureus alpha toxin (AT) on selectin mediated PMN adherence and vasoactivity in rat aortic rings. Adherence of human PMNs to rat aortic endothelium increased significantly following stimulation with AT (0.1, 0.5, and 1 μg/ml). However, neutrophil adherence was significantly attenuated by fucoidin known to block P- and L-selectin. Unstimulated

human PMNs (10^6cells/mL) were added to organ chambers containing rat aortic rings stimulated with AT (0.1 and 0.5 µg/ml). PMNs elicited a significant vasocontraction in AT stimulated rat aortic rings (46±6, 88±7 mg, $p<0.05$). This PMN-induced vasocontraction was significantly attenuated by pretreatment with fucoidin ($p<0.05$). Endothelial function as assessed by endothelium-dependent vasorelaxation to acetylcholine was also significantly attenuated after PMN incubation with AT stimulated rat aortic rings. This endothelial dysfunction was significantly reduced by fucoidin. In contrast, endothelium-independent relaxation was not altered by PMN incubation, indicating that vascular smooth muscle function was unaffected.

Thus, PMN-endothelium interaction following Staphylococcus aureus alpha toxin activation mediated by selectin adhesion molecules (ie, P-selectin and L-selectin) may play an important role in PMN-induced vasocontraction and endothelial dysfunction. This mechanism may be important in early endothelial dysfunction observed following septicemia.

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Protective effects of *n*-acetylcysteine in a rat model of splanchnic artery occlusion and reperfusion

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The aim of the present study was to investigate the beneficial effect of *n*-acetylcysteine (NAC) in a model of splanchnic artery occlusion shock (SAO). SAO shock was induced in rats by clamping both the superior mesenteric artery and the celiac trunk for 45 min, followed thereafter by release of the clamp (reperfusion). At 60 minutes after reperfusion, animals were sacrificed for histological examination and biochemical studies. SAO shocked rats developed a significant fall in mean arterial blood pressure, significant increase of tissue myeloperoxidase and malonaldehyde activity, and marked histological injury to the distal ileum. SAO shock was also associated with a significant mortality (0% survival at 2 h after reperfusion). Reperfused ileum tissue sections from SAO-shocked rats showed positive staining for P-selectin and for intercellular adhesion molecules (ICAM-1). NAC (applied at 100 mg/kg, 5 min prior to reperfusion, followed by an infusion of 100 mg/kg h), significantly reduced ischemia/reperfusion injury in the bowel as evaluated by histological examination, reduced myeloperoxidase and malonaldehyde activity, and markedly reduced the intensity and degree of P-selectin and ICAM-1, reduced the fall in mean arterial blood pressure and improved survival. Our results clearly demonstrate that NAC treatment exert a protective effect and part of this effect may be due to inhibition of the expression of adhesion molecules and subsequent reduction of neutrophil-mediated cellular injury.

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Protective effects of melatonin in a rat model of splanchnic artery occlusion and reperfusion

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The aim of the present study was to investigate the protective effect of the pineal hormone melatonin in a model of splanchnic artery occlusion shock (SAO). SAO shock was induced in rats by clamping both the superior mesenteric artery and the celiac trunk for 45 min, followed thereafter by release of the clamp (reperfusion). At 60 minutes after reperfusion, animals were sacrificed for histological examination and biochemical studies. Immunohistochemical examination demonstrated a marked increase in the immunoreactivity to nitrotyrosine, an index of nitrogen species such as peroxynitrite, in the necrotic ileum in shocked rats. SAO shocked rats developed a significant increase of tissue myeloperoxidase and malonaldehyde activity, and marked histological injury to the distal ileum. SAO shock was also associated with a significant mortality (0% survival at 2 h after reperfusion). Reperfused ileum tissue sections from SAO-shocked rats showed positive staining for P-selectin, which was mainly localised in the vascular endothelial cells. Ileum tissue section obtained from SAO-shocked rats with anti-intercellular adhesion molecules (ICAM-1) antibody showed a diffuse staining. Melatonin, (applied at 3 mg/kg, 5 min prior to reperfusion, followed by an infusion of 3 mg/kg h), significantly reduced ischemia/reperfusion injury in the bowel as evaluated by histological examination, prevented the infiltration of neutrophils into the reperfused intestine, as evidenced by reduced myeloperoxidase activity, reduced lipid peroxidation, as evaluated by malonaldehyde activity, markedly reduced the intensity and degree of P-selectin and ICAM-1 in tissue section from SAO-shocked rats and improved survival. Taken together, our results clearly demonstrate that melatonin treatment exert a protective effect and part of this effect may be due to inhibition of the expression of adhesion molecules and subsequent reduction of neutrophil-mediated cellular injury.

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EFFECT OF MONOCLONAL ANTIBODY LFA-1 ON LEUKOCYTE ADHERENCE FOLLOWING HEMORRHAGIC SHOCK IN RATS.

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The activation and adherence of leukocytes to the venular endothelium is a critical step in the pathogenesis of generalized microvascular injury following hemorrhagic shock. Previous studies have shown that the integrins CD11/CD18 play a significant role in this interaction. The purpose of this study was to examine; 1) anti-LFA-1, an antibody to CD11a/CD18, in attenuating leukocyte adherence following hemorrhagic shock, and 2) the efficacy of using anti-LFA-1 as pretreatment (anti-LFA-1 10min before shock), during shock (anti-LFA-1 20min into shock), and resuscitation (anti-LFA-1 10 min post shock). Following a control period, blood was withdrawn to reduce the mean arterial pressure to 40 mmHg for 30 minutes in urethane-anesthetized rats. A transilluminated segment of the mesentery was examined to quantitate leukocyte adherence using intravital microscopy. Anti-LFA-1 significantly attenuated leukocyte adherence before, during and partially after hemorrhagic shock.

The results are shown below:

	Leukocyte Adherence cell/100μm			
	Before shock	After shock (resuscitation)		
		10min	30min	60min
Control n=5	0.5	3.8	6.7	9.4
Anti-LFA-1 Pretreatment n=5	0.6	1.1*	0.9*	1.1*
Anti-LFA-1 Shock n=5	0.8	2.0*	1.5*	1.7*
Anti-LFA-1 Resuscitation n=5	1.7	5.2	5.1	5.8*

*Indicates $p<0.05$

Anti-LFA-1 appears to offer almost complete protection against leukocyte adherence when given before and during shock. This protection can be even demonstrated at10min post shock. These results suggest that anti-LFA-1 may be of potential therapeutic benefit against microvascular injury caused by hemorrhagic shock.

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HEPATIC E-SELECTIN EXPRESSION AND ITS FUNCTIONAL SIGNIFICANCE DURING ENDOTOXIN-INDUCED LIVER INJURY. H. Jaeschke, J.A. Lawson*, A.R. Burns*, A. Farhood*, and C.W. Smith*. Pharmacia & Upjohn, Inc. Kalamazoo, MI; 49007, Baylor College of Medicine and UT Health Science Center, Houston, TX, 77030, Univ of Arkansas for Med Sciences, Little Rock, AR, 72205.

Neutrophils can cause parenchymal cell injury in the liver during ischemia-reperfusion and endotoxemia. Neutrophils relevant for the injury accumulate in sinusoids, transmigrate and adhere to hepatocytes (Am J Physiol 272:G1195-G1200,1997). To investigate the role of E-selectin in this process, male C3Heb/FeJ mice were treated with 700 mg/kg galactosamine and 100 μg/kg endotoxin (Gal/ET). Immuno-histochemical analysis indicated no E-selectin expression on sinusoidal lining cells in control livers. However, 3 and 5 h after Gal/ET, there was increased E-selectin expression on sinusoidal and venular endothelium. Gal/ET induced hepatic neutrophil accumulation (422 ± 32 PMN/50 high power fields) and severe liver injury (plasma ALT activities: 4120 ± 960 U/L; necrosis: 44 ± 3%) at 7 hours. Approximately 30-35% of the neutrophils entered the parenchyma. Intravenous administration (3 mg/kg) of an E-selectin antibody (clone 10E9.6) had no significant effect on neutrophil accumulation in the vasculature (472 ± 26 PMN/50 HPF) or extravasation. However the E-selectin antibody significantly attenuated liver injury as indicated by lower ALT levels (670 ± 200 U/L) and 43% less necrotic hepatocytes (area of necrosis: 25 ± 4%). In contrast, an isotype-matched control antibody did not affect hepatic neutrophil sequestration, extravasation or liver injury. Conclusion: Endotoxin induces E-selectin expression on sinusoidal lining cells. Sinusoidal E-selectin does not appear to be involved in neutrophil localization in the liver. However, because in vitro experiments indicate that E-selectin engagement can activate neutrophils, the protective effect of the E-selectin antibody in vivo suggests that E-selectin may further activate neutrophils during transmigration thereby facilitating the adherence and attack on parenchymal cells

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DEFICIENCY OR FUNCTIONAL INHIBITION OF P-SELECTIN ATTENUATES LEUKOCYTE-ENDOTHELIUM INTERACTION INDUCED BY HEMORRHAGE AND REINFUSION. Rosario Scalia*, Valerie E. Armstead*, Alexander G. Minchenko* and Allan M. Lefer. Department of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

The role exerted by P-selectin in the recruitment of leukocytes during hemorrhage was investigated in wild-type and P-selectin deficient mice. Mice were hemorrhaged by withdrawal of blood to a MABP of 40 mmHg for 45 minutes. Mice were then resuscitated by infusion of the shed blood and intravenous injection of 0.5 ml 0.9% NaCl alone or with either an anti-P-selectin monoclonal antibody or a recombinant soluble form of PSGL-1. Leukocyte-endothelium interactions were studied in peri-intestinal venules by means of intravital microscopy. Resuscitation from hemorrhage increased significantly the number of rolling and adherent leukocytes in the splanchnic microcirculation of wild-type mice, as well as the number of leukocytes that infiltrated into lung, liver and intestine ($p<0.01$ vs sham operated control mice). A significant increase in P-selectin expression on the microvascular endothelium of hemorrhaged wild-type mice occurred ($p<0.01$ vs sham operated control mice). In contrast, mice genetically deficient in P-selectin, or wild-type mice given either an anti-P-selectin monoclonal antibody or a recombinant soluble PSGL-1 immunoglobulin, exhibited markedly attenuated leukocyte-endothelium interaction, following hemorrhage and reinfusion. In addition, endogenous levels of PSGL-1 mRNA were significantly increased in the lung, liver and intestine of wild-type mice subjected to hemorrhage and reinfusion. Since PSGL-1 promotes adhesive interactions largely through P-selectin expressed on the vascular endothelium, these findings further support the essential role played by P-selectin in the recruitment of leukocytes during hemorrhagic shock.

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EXPRESSION OF CYTOKINES IN LUNG INJURY AFTER INTERVENTION WITH ANTI-L-SELECTIN IN EXPERIMENTAL ISCHEMIA-REPERFUSION. M. van Griensven*, M. Stalp*, U. Lehmann* and A. Seekamp. Department of Trauma Surgery, Hannover Medical School, D-30625 Hannover, Germany

This study was performed to investigate the influence of anti-L-selectin on cytokine mRNA expression in lung and muscle tissue and on secondary lung injury after ischemia-reperfusion. L-selectin plays a key role in the so-called „rolling" of PMN. To induce ischemia, the abdominal aortae of 10 Merino sheep were infrarenally ligated for three hours. Subsequently, the ligature was removed and a reperfusion phase of 4 hours was initiated. An infusion of anti-L-selectin (EL-246 1 mg/kg) was performed during the first 15 minutes of reperfusion. As a positive control (POS), an irrelevant antibody was applied to the sheep (n=10). Ten sheep not undergoing ischemia, serve as

negative controls (NEG). Intervention with anti-L-selectin resulted in a decreased expression of IL-1, IL-6 and TNFα in lung tissue in comparison to POS as measured by a competitive RT-PCR. In all groups, no mRNA expression of the cytokines was detected in muscle tissue. Myeloperoxidase (MPO) showed highest activity (31.7 U/g tissue) in POS. An increased BAL to plasma protein ratio (0.76) could be determined. These results were significantly higher in comparison to the sheep receiving anti-L-selectin ($p<0.01$). In this group, MPO activity amounted to 10.2 U/g and the protein ratio to 0.42. Again both groups showed significantly higher levels in comparison to NEG. Intervention with anti-L-selectin resulted in a reduced MPO activity of 84% ($p<0.01$) and a reduced protein ratio of 59% ($p<0.01$) in comparison to POS. Thus, anti-L-selectin reduces the pulmonary diapedesis of PMN and may therefore positively influence ischemia-reperfusion injury.

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SENSORY NEUROPEPTIDE CGRP, BUT NOT SP, POTENTIATES LPS-INDUCED CHEMOKINE KC SECRETION FROM THE MONO/MACROPHAGES IN MURINE SEPSIS. X. Wang[#], D. Call[*], S. Ebong[*], D. Newcomb[*], G. Bolgos[*] and D. Remick.
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Previous publications in ours and other lab demonstrated that the systemic levels of sensory neuropeptides, calcitonin gene-related peptide (CGRP) and substance P (SP), and CXC chemokine KC are increased in endotoxin and septic shock in animals. It was reported that CGRP and SP are chemotactic for neutrophils. We tested whether these sensory neuropeptides could modulate KC secretion from three sources of the mono/macrophages in murine sepsis. Mono/macrophages were obtained from the peritoneal exudate, spleen and lung of male Balb/c mouse. The cells were plated on culture dishes with CGRP/SP and/or LPS 0.1 μg/ml for 12 hrs post-16 hrs CLP with a 21 gauge needle. The KC level in medium was measured by ELISA assay. The results showed that CGRP (0.1-10 nM) significantly potentiated LPS-induced KC production from peritoneal and spleen, but not lung, mono/macrophages in a concentration-dependent manner in CLP subjects. In contrast, no enhancement was observed in normal subjects. SP, colocalized with CGRP had no effects on both normal and CLP subjects. These results suggest that sensory neuropeptide CGRP, but not SP appears to be intimately involved in chemotactic for neutrophils by promoting the chemokine KC in murine sepsis. CGRP appears to have the capacity to the modulate inflammatory responses in sepsis.

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A NOVEL ANTI-APOPTOTIC AGENT (LXR-1035) INHIBITS LEUKOCYTE-ENDOTHELIUM INTERACTION *IN VIVO*. D. Pruefer*, R. Scalia* and A.M. Lefer. Dept of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

We studied the effect of LXR-1035, a novel lysophosphatidic acid formulation, on leukocyte-endothelial cell interaction in the rat mesenteric microvasculature. Superfusion of the rat mesentery with 50 μM L-NAME significantly increased leukocyte rolling, adherence, and transmigration compared to control mesenteries superfused with Krebs-Henseleit (K-H) solution. However, superfusion of the rat mesentery with LXR-1035 (300 nM) consistently inhibited the L-NAME-induced leukocyte rolling (47±4 vs. 10±2 cells/min, $p<0.01$), adherence (17±3 vs. 3±1 cells/100 μm length of venule, $p<0.01$), and trans-migration (8±2 vs. 1.5±0.5 cells/100 μm x 20 μm, $p<0.01$), without altering systemic blood pressure or mesenteric venular shear rate. Similar results were also obtained in rats subjected to 90-min hemorrhage followed by 90-min reperfusion. Resuscitation from hemorrhage significantly increased the number of rolling, adherent and transmigrating leukocytes in K-H superfused rat mesenteries. However, superfusion of the rat mesentery with LXR-1035 markedly attenuated leukocyte-endothelium interaction, following hemorrhage and reinfusion. Immunohistochemical localization of P-selectin expression on mesenteric venules was significantly increased ($p<0.01$) after exposure to L-NAME or following hemorrhage-reinfusion, which was significantly attenuated by LXR-1035 ($p<0.05$). Thus, LXR-1035 potently inhibits recruitment of leukocytes into inflamed tissues, by down-regulating P-selectin expression.

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*Numbers refer to abstract numbers, not page numbers.